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PULMONARY FUNCTION STUDIES IN PATIENTS WITH MITRAL STENOSIS[1, 2]

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Introduction

Clinical and pathologic pulmonary changes associated with mitral stenosis are well known (1–7). Such early symptoms of mitral stenosis as dyspnea, cough, and wheezing reflect impaired pulmonary function. Increased pulmonary interstitial connective tissue and variable degrees of edema may result from chronically high pressure transmitted from the left atrium. In addition, vascular changes include thickening of the capillary basement membrane, intimal thickening and, probably, muscular hypertrophy. Altered pulmonary physiology as measured by various pulmonary function tests has been described by many workers (6, 8–19). Attempts to correlate such changes to the functional classification (8, 10), and to vessel and interstitial tissue changes in lung biopsies obtained during cardiac catheterization (3, 5, 6) have been generally inconclusive.

It is the purpose of this paper to report: ventilatory studies in a group of 45 patients with predominant mitral stenosis, including correlations with hemodynamic data; alveolar air and arterial blood gas tensions in a separate group of 24 patients with the same diagnosis; and diffusing capacity for oxygen and venous admixture in 18 patients.

Materials and Methods

All of the patients were categorized according to the functional classification adopted by the New York Heart Association (20). The first group included 45 patients, 17 males and 28 females, ranging in age from twenty-four to fifty-six years. Pulmonary function studies and cardiac catheterization were carried out on separate days, usually less than a week apart. Pulmonary function tests included partitioning the total lung volume by the helium-dilution method described by Meneely and Kaltreider (21). Oxygen consumption and minute ventilation were obtained by spirometer recording during quiet breathing of 100 per cent oxygen. The maximal breathing capacity and timed vital capacity were measured with a low-resistance 13.5-liter Collins respirometer. Observed lung volume and maximal breathing capacity were compared with predicted normal values according to the regression equations of Baldwin and associates (22) and Motley (23), respectively. Additional calculations included air velocity index (the ratio between the predicted vital capacity and the maximal breathing capacity) and ventilatory equivalent for oxygen (minute ventilation in liters per 100 cc. oxygen uptake). Cardiac catheterization was carried out on each patient two to three hours following a light breakfast. The details of this procedure have been reported (24). The lower limit of arterial blood oxygen saturation in normal subjects in this laboratory is 93 per cent.

Total pulmonary and pulmonary vascular resistances were calculated according to the formulas described by Gorlin and co-workers (25).

The second group consisted of 24 patients, 10 males and 14 females, ranging in age from twenty-four to fifty-three years. Hemodynamic studies,

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including alveolar air and arterial blood gas analysis on these patients during the breathing of ambient air and of 12 per cent oxygen in nitrogen, have been reported in a previous publication (26). Sufficient data were obtained from 18 patients for the determination of the diffusing capacity for oxygen and venous admixture according to the method described by Riley and coworkers (27). Arterial blood oxygen and carbon dioxide tensions were determined by the method of Riley and associates (28), and the partial pressure of alveolar oxygen was calculated from the equation described by Pappenheimer *et al.* (29).

In normal subjects, the lower limit of the diffusing capacity for oxygen was calculated to be 9 ml. per minute per mm. of mercury (13), and the ratio of the venous admixture or physiologic shunt to the cardiac output was less than 7 per cent (30).

Results

Data from the ventilatory studies performed on the 45 patients of the first group are arranged according to functional classification in table 1. The oxygen tensions of alveolar air and arterial blood in patients of the second group similarly classified are presented in table 2. The pressures and resistances in the pulmonary circuit and diffusing capacity for oxygen and venous admixture expressed as percentage of cardiac output in 18 patients of the second group are shown in table 3.

TABLE 1

Ventilatory Studies and Arterial Blood Oxygen Saturation in 45 Patients With Mitral Stenosis

	Functional Classes					
	II (5 Cases)		III (31 Cases)		IV (9 Cases)	
	Range	Mean	Range	Mean	Range	Mean
I. Ventilatory studies						
A. Subdivisions of lung volumes						
Vital capacity (per cent of predicted)	63–109	94	50–141	90	63–114	81
Residual volume (per cent of predicted)	109–217	146	79–272	166	101–259	174
Total lung capacity (per cent of predicted)	73–130	104	64–164	111	86–149	106
RV/TLC × 100	25–35	31	21–63	36	31–49	40
B. Timed vital capacity (per cent of total capacity)						
1-second	55–92	78	58–86	77	55–84	70
2-second	75–99	90	71–95	90	77–92	88
3-second	86–100	95	76–100	95	86–100	95
C. Maximal breathing capacity (per cent of predicted)	103–108	105	43–119	80	55–104	76
D. Air velocity index	1.0–1.1	1.05	0.51–1.29	0.86	0.60–1.10	0.85
E. Breathing reserve at rest (per cent of MBC)	94–95	95	80–95	91	86–93	89
II. Ventilation and arterial blood oxygen saturation at rest						
A. Minute ventilation (L. per min. per M.²)	3.54–5.68	4.46	2.94–6.52	4.16	3.42–7.29	5.13
B. Minute oxygen uptake (cc. per min. per M.²)	116–157	140	116–171	141	130–170	156
C. Ventilatory equivalent (L. per 100 cc. of oxygen)	2.0–2.9	2.5	1.9–3.1	2.2	1.9–4.6	3.0
D. Arterial blood oxygen saturation (per cent)	90.6–93.8	92.7	74.7–100.3	93.8	82.0–99.7	91.2

TABLE 2

Alveolar and Arterial Blood Gas Analysis in 24 Patients With Mitral Stenosis

	Functional Classes					
	II (12 Cases)		III (8 Cases)		IV (4 Cases)	
	Range	Mean	Range	Mean	Range	Mean
A. Alveolar oxygen tension (P_{AO_2}) (mm. Hg)	97–110	104	86–110	100	95–105	101
B. Arterial blood oxygen tension (P_{aO_2}) (mm. Hg)	50–93	78	46–98	74	58–75	68
C. P_{AO_2}—P_{aO_2} gradient (mm. Hg)	3–45	26	12–53	27	30–41	34
D. Arterial blood carbon dioxide tension (P_{aCO_2}) (mm. Hg)	28–45	38	32–53	40	37–49	42

Vital capacity: In this study, vital capacity is expressed as per cent of predicted normal. Mean values in the functional categories were lower in the more severely affected groups. All but one patient in *Class II* had vital capacities of 98 per cent or more of predicted normal. One-third of those in *Class III* and two-thirds of *Class IV* had vital capacities of 85 per cent or less. These values were apparently unrelated to age. The mean values for the three classes of patients were 94 per cent, 90 per cent, and 81 per cent.

As shown in figure 1, the mean per cent of predicted normal for vital capacity declined in relation to increasing duration of dyspnea. The categories for years of dyspnea presented were arbitrarily chosen. It is notable that the dyspnea of 41 patients was nearly evenly distributed in duration between one and ten years. Only 4 patients had had dyspnea for less than one or more than ten years. These findings are in agreement with those reported by other writers (9). Negative correlation was observed between vital capacity and pulmonary arterial mean pressure ($r = -0.39$; $p = 0.005$); total pulmonary resistance ($r = -0.49$; $p = 0.01$); and pulmonary vascular resistance ($r = -0.43$; $p = 0.05$). While these correlations were all statistically significant, there was really no predictive relationship in any case. No correlation was demonstrated between the vital capacity and residual volume, air velocity index, pulmonary "capillary" mean pressure, mitral valve area, or cardiac index.

Residual volume: The values for residual volume are also expressed as per cent of predicted normal. Patients in *Class II* showed a range of 109 to 217 per cent and a mean of 146 per cent. In *Class III*, the range was 79 to 272 per cent and the mean, 166 per cent. The range was 101 to 259 per cent in *Class IV* with a mean of 174 per cent. Although there was considerable variation in values observed in each class, the increase in mean values from *Classes II* to *IV* was consistent. The total lung capacity remained remarkably constant throughout.

There was no correlation between residual volume and any of the following hemodynamic determinants: pulmonary arterial mean pressure, pulmonary "capillary" mean pressure, total pulmonary resistance, pulmonary vascular resistance, mitral valve area, or cardiac index.

Timed vital capacity: Timed vital capacity is expressed as per cent of total vital capacity at each second for a total of three seconds. As

TABLE 3

PRESSURES AND RESISTANCES IN THE PULMONARY CIRCUIT, DIFFUSING CAPACITY FOR OXYGEN, AND VENOUS ADMIXTURE IN 18 PATIENTS WITH MITRAL STENOSIS

Patient	Functional Classification	PA_m	"PC"$_m$	TPR	PVR	D_{O_2}/M^2	$\frac{Q_{va}}{Q_t} \times 100$
A. E.	II	22*	12	380	180	13.5	16
		27†	10	570	360		
V. J.	II	28	14	780	390	10.6	7
		50	8	1,400	1,170		
G. W.	II	28	20	310	90	13.2	11
		40	27	400	120		
J. G.	II	29	14	270	190	6.3	4
		30	14	330	210		
V. P.	II	29	—	250	—	15.3	7
		44	—	430	—		
W. G.	II	30	15	300	150	N‡	14
		43	18	600	380		
J. R.	II	30	29	470	90	10.6	21
		40	—	—	—		
J. B.	II	33	17	480	230	6.3	4
		36	—	590	—		
M. S.	II	33	25	470	110	N	2
		42	26	680	280		
H. D.	II	35	24	330	120	6.0	3
		41	21	170	80		
B. O.	III	19	14	330	200	6.9	3
		29	—	460	—		
A. Et.	III	33	22	650	220	7.2	5
		44	—	570	—		
N. C.	III	46	34	1,040	270	N	22
		61	—	1,320	—		
M. K.	III	56	32	1,110	480	7.1	10
		64	—	1,290	—		
J. A.	III	73	30	1,500	880	9.6	5
		84	31	1,550	980		
F. L.	IV	50	33	1,040	350	7.0	3
		54	—	750	—		
L. R.	IV	61	34	910	380	5.8	14
		75	—	920	—		
S. N.	IV	80	—	1,730	—	7.2	10
		97	—	1,620	—		

PA_m = pulmonary artery mean pressure (mm. Hg.)

"PC"$_m$ = pulmonary artery wedge pressure (mm. Hg.)

TPR = total pulmonary resistance (dynes-sec. per cm.^{5})

PVR = pulmonary vascular resistance (dynes-sec. per cm.^{5})

D_{O_2}/M^2 = diffusing capacity for oxygen (ml. per min. per mm. Hg.)

$\frac{Q_{va}}{Q_t} \times 100$ = venous admixture as percentage of cardiac output.

* The upper paired values represent observations during the breathing of ambient air.

† The lower values represent observations during the breathing of 12 per cent oxygen in nitrogen.

‡ Diffusing capacity for oxygen is expressed as normal (N) when the mean alveolar—capillary oxygen tension is small.

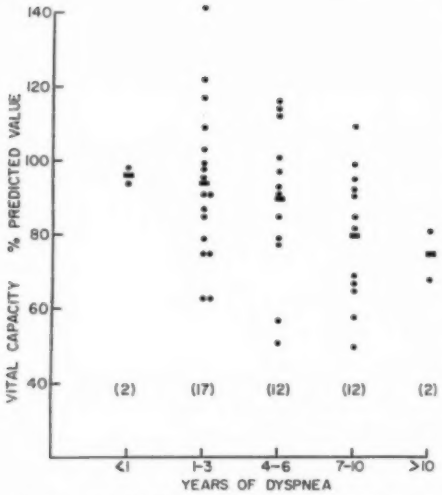

FIG. 1. Graph illustrating the relationship between vital capacity and duration of dyspnea in 45 patients with mitral stenosis. In this and subsequent graphs, the figures in parentheses indicate the number of patients. The bar in each column is the mean value, and each solid circle represents one patient.

shown in table 1, no significant change in mean values was observed among functional classes.

Maximal breathing capacity: This is expressed as per cent of predicted normal. In this study, the mean values for each functional class are as follows: *Class II*, 105 per cent; *Class III*, 80 per cent; and *Class IV*, 76 per cent. All of the patients of *Class II* had a maximal breathing capacity greater than 100 per cent. One-third of the total number of patients in *Classes III* and *IV* had maximal breathing capacities of 65 per cent or less.

There was no demonstrable correlation between the maximal breathing capacity and the six hemodynamic determinants.

Air velocity index: In each of the three functional classes, the air velocity index was consistently well within the range of normal. No correlation existed between the air velocity index and the six hemodynamic determinants.

Breathing reserve at rest: The mean values for breathing reserve at rest in functional *Classes II* to *IV* were 95 per cent, 91 per cent, and 89 per cent. The values observed for the 5 patients in *Class II* were 94 to 95 per cent, whereas 4 of the 31 patients in *Class III* and 2 of the 9 patients in *Class IV* had breathing reserves below 87 per cent. The range for *Class III* was 80 to 95 per cent, and that for *Class IV*, 86 to 93 per cent.

Minute ventilation, minute oxygen uptake, and ventilatory equivalent for oxygen: The minute ventilation for functional *Classes II* and *III* was 4.46 and 4.16 L. per min. per M.^{2} Corresponding mean values for oxygen uptake were 140 and 141 cc. per min. per M.^{2} The efficiency of oxygen extraction for these two functional classes, reflected in the ventilatory equivalent for oxygen, was 2.5 and 2.2 L. per 100 cc. of oxygen. In functional *Class IV*, mean values for minute ventilation and minute oxygen uptake were, respectively, 5.13 L. per min. per M.^{2} and 156 cc. per min. per M.^{2} The ventilatory equivalent for this class was slightly higher (3.0 L. per 100 cc. of oxygen) than that in *Classes II* and *III*.

Arterial blood oxygen saturation: The mean values for arterial blood oxygen saturation in patients of the first group during cardiac catheterization were 92.7 per cent for *Class II*, 93.8 per cent for *Class III*, and 92.1 per cent for *Class IV*. The mean values of the arterial blood oxygen saturation of the 24 patients in the second group were as follows: *Class II*, 92 per cent; *Class III*, 89 per cent; and *Class IV*, 85 per cent. Arterial blood oxygen saturation failed to correlate with the six hemodynamic determinants.

Alveolar air and arterial blood gas tension: As shown in table 2, during the breathing of ambient air the mean alveolar air oxygen tension ($P_{A_{O_2}}$) was within normal for the 24 patients of the second group. On the other hand, the mean arterial blood oxygen tension ($P_{a_{O_2}}$) was uniformly decreased (78, 74, and 68 mm. of mercury for the three classes, respectively). Considerable variation was encountered among patients in each functional class for alveolar air and arterial blood oxygen tensions. Each of the 4 patients in *Class IV* had a subnormal $P_{a_{O_2}}$ (58–75 mm. of mercury). The mean $P_{A_{O_2}}$—$P_{a_{O_2}}$ gradient was significantly widened in all three classes (26, 27, and 34 mm. of mercury) (figure 2). The gradient was more than 12 mm. of mercury, the upper limit of normal, in all but one patient. All 4 patients of *Class IV* had gradients higher than the mean of either *Class II* or *Class III* (30 to 41 mm. of mercury). The mean arterial blood carbon dioxide tension ($P_{a_{CO_2}}$) was within normal limits for all three classes (38, 40, and 42 mm. of mercury), although slight elevations were noted in several patients in each.

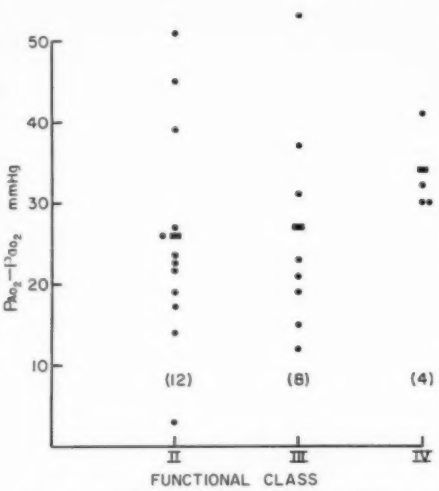

FIG. 2. Graph illustrating the P_{AO_2}—P_{aO_2} gradient in 24 patients with mitral stenosis classified according to functional disability.

There was no significant correlation between the P_{AO_2}—P_{aO_2} gradient and pulmonary arterial mean pressure, pulmonary "capillary" pressure, total pulmonary resistance, pulmonary vascular resistance, and right ventricular work.

Diffusing capacity for oxygen and venous admixture: The diffusing capacity for oxygen (D_{O_2}) and venous admixture as percentage of the cardiac output ($Q_{va}/V_t \times 100$) were measured in 18 patients. The data are presented in table 3. Subnormal D_{O_2} was observed in 3 of 10 patients of functional *Class II*, in 2 of 5 patients of *Class III*, and in all 3 patients of *Class IV*. In general, the more disabled the patients, the higher the pulmonary arterial pressure and the more marked the impaired diffusing capacity for oxygen.

However, the venous admixture expressed as percentage of the cardiac output varied considerably in patients of different functional classes. No consistent relationship was observed between the per cent of venous admixture and the magnitude of the pulmonary hypertension.

DISCUSSION

This study showed that the most severely dyspneic of the patients with mitral stenosis are those whose vital capacities are most diminished. More than three-fourths of the patients in *Class IV* and about half of the patients with dyspnea of four or more years' duration had significant reduction in vital capacity. The pathologic changes in the lungs, including edema, fibrosis, and reduction in the number of functional vessels, may be responsible for incapacity in the more advanced cases.

In addition, the work of breathing has been shown to be definitely increased, particularly on exercise, in many cases of mitral stenosis; this is presumably due to increased rigidity of the lungs (14).

The increase in the residual volume appeared to occur at the expense of the vital capacity, especially in patients of *Class IV*; hence, the average total lung capacity in the three classes of patients was normal or slightly increased. However, there was a progressive increase in the mean values of the ratio of the residual volume to total lung capacity (RV/TLC) from patients in *Class II* to those in *Class IV*.

The results observed for timed vital capacity, maximal breathing capacity, and air velocity index suggest that an obstructive phenomenon does not contribute significantly to the ventilatory insufficiency of most of the patients. This is in agreement with the results reported by Frank and his co-workers (10). In addition, they found that there was no evidence of impairment of intrapulmonary mixing in their patients.

MacIntosh and associates (17) found that there was a progressive elevation of the ventilatory equivalent in patients with mitral stenosis from *Class I* to *Class IV*, with no overlapping of the values between patients in *Class II* and *Class IV*. The present observations were at variance with their results in that there was no consistent increase in the ventilatory equivalent in patients from *Class II* to *Class IV*.

The alveolar air oxygen and carbon dioxide tensions were within normal, but the arterial blood oxygen tension was markedly decreased. The observation of a widened P_{AO_2}—P_{aO_2} gradient in this study is in accord with similar observations reported by other workers (9, 13, 15, 16).

In half of the 18 patients, the diffusing capacity for oxygen was considered to be subnormal (13). The low value of diffusing capacity for oxygen was observed more frequently in patients of *Classes III* and *IV* than in those of *Class II*. This finding was in agreement with that reported by MacIntosh and associates (17). Possible mechanisms for the low diffusing capacity, such as abnormalities in the character of diffusing surface

or reduction in the total effective diffusing area, were discussed by Carroll and co-workers in detail (13). It is likely that the presence of pulmonary congestion, pulmonary fibrosis, or closure of pulmonary capillaries may be important factors.

MacIntosh and associates (17) reported that there was no significant improvement in diffusing capacity of the lungs in a group of patients with mitral stenosis studied up to a period of eighteen months following mitral commissurotomy.

Summary

Pulmonary function studies were conducted in 45 patients with mitral stenosis. An attempt was made to correlate the results with hemodynamic determinants measured during cardiac catheterization.

Among the pulmonary function tests investigated, the vital capacity appeared to be most closely related to clinical deterioration. A negative correlation was observed between vital capacity and pulmonary arterial mean pressure, total pulmonary resistance, and pulmonary vascular resistance, but there was no predictive relationship in any case. The vital capacity was shown to diminish with increasing duration of dyspnea.

Mean values for residual volume were shown to increase progressively with function class. This increase apparently occurred in association with a reduction in vital capacity and little change in total lung capacity. No significant correlation was found between the residual volume and the hemodynamic determinants.

The maximal breathing capacity and other pulmonary function tests changed only slightly and not in proportion to changes in clinical status. No significant correlation with hemodynamic determinants was found.

Alveolar air and arterial blood oxygen tension and gradient during the breathing of ambient air were calculated for a separate group of 24 patients. The gradient was significantly widened in all three functional classes, primarily because of subnormal arterial blood oxygen tension.

Subnormal diffusing capacity for oxygen was observed in 9 of 18 patients. The low values were found more frequently in patients of *Classes III* and *IV* than in those of *Class II*.

Venous admixture expressed as percentage of cardiac output varied considerably in patients of different classes and was not related to magnitude of pulmonary hypertension.

Sumario

Estudios de la Función Pulmonar en Enfermos de Estenosis Mitral

Estos estudios de la función pulmonar fueron llevados a cabo en 45 enfermos de estenosis mitral. Tratóse de correlacionar los resultados con las determinaciones hemodinámicas verificadas durante el cateterismo cardíaco.

Entre las pruebas de la función pulmonar ejecutadas, la de la capacidad vital pareció ser la aliada más íntimamente con el deterioro clínico. Observóse una correlación negativa entre la capacidad vital y la tensión arteriopulmonar media, la resistencia pulmonar total y la resistencia vasculopulmonar, pero sin poderse predecir la relación en ningún caso. Notóse que disminuía la capacidad vital al aumentar la duración de la disnea. Según se observó, los valores medios del volumen residual aumentaban progresivamente con la clase de la función. Este aumento se asociaba aparentemente con una reducción de la capacidad y poco cambio de la capacidad pulmonar total. No se descubrió mayor correlación entre el volumen residual y las determinaciones hemodinámicas.

Las pruebas de la capacidad respiratoria máxima y de otras funciones pulmonares apenas se alteraron, sin guardar relación con las modificaciones del estado clínico. No se descubrió mayor correlación con las determinaciones hemodinámicas.

Para otro grupo de 24 enfermos se calcularon la tensión y la pendiente del aire alveolar y del oxígeno de la sangre arterial durante la respiración de aire ambiente. La pendiente estaba ensanchada significativamente en las tres clases de función, debido primordialmente a una tensión subnormal del oxígeno de la sangre arterial.

Observóse una capacidad difusora subnormal para el oxígeno en 9 de 18 enfermos. Las cifras bajas correspondieron más frecuentemente a los enfermos de las clases III y IV que a los de la clase II.

La mezcla venosa expresada en forma de porcentaje del rendimiento cardíaco varió considerablemente en los enfermos de las distintas clases, sin relacionarse con la magnitud de la hipertensión pulmonar.

Resume

Étude de la fonction pulmonaire chez des malades atteints de sténose mitrale

Une étude de la fonction pulmonaire a été effectuée chez 45 malades atteints de sténose mitrale. On a tenté d'établir une corrélation des résultats avec les facteurs déterminants hémo-

dynamiques mesurés durant le cathétérisme cardiaque.

Parmi les tests de la fonction pulmonaire étudiés, c'est la capacité vitale qui paraissait être le plus étroitement liée aux altérations cliniques. Une corrélation négative a été observée entre la capacité vitale et la pression artérielle pulmonaire moyenne, la résistance pulmonaire total et la résistance vasculaire pulmonaire, mais dans aucun cas la corrélation ne pouvait être prévisible. Il a été démontré que la capacité vitale diminuait suivant l'augmentation de durée de la dyspnée.

Les valeurs moyennes du volume résiduel ont présenté une augmentation progressive selon la classe fonctionnelle. Cet accroissement paraissait associé à la réduction de la capacité vitale avec une modification insignificante de la capacité pulmonaire totale. Aucune corrélation notable n'a été observée entre le volume résiduel et les facteurs déterminants hémodynamiques.

La capacité respiratoire maximale et autres épreuves de la fonction pulmonaire ne subissaient que de légères variations, sans proportion avec les modifications de l'état clinique. Aucune corrélation significative avec les facteurs déterminants hémodynamiques n'a été observée.

L'air alvéolaire et la tension de l'oxygène du sang artériel, et le gradient durant la respiration de l'air ambiant, ont été déterminés dans un groupe séparé comprenant 24 patients. Le gradient était notablement augmenté dans les trois places fonctionnelles, surtout en raison de la tension subnormale de l'oxygène du sang artériel.

La capacité de diffusion subnormale de l'oxygène a été observée chez 9 malades sur 18. Les valeurs basses ont été plus fréquemment rencontrées chez les malades des classes III et IV que chez ceux de la classe II.

Le mélange veineux exprimé en pourcentage de débit cardiaque variait considérablement chez les malades des différentes classes et n'était pas en relation avec l'importance de l'hypertension pulmonaire.

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HOSPITAL ADJUSTMENT OF TUBERCULOUS PATIENTS ON DIFFERENT WARDS OF THE SAME HOSPITAL[1]

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Introduction

Hospital adjustment of tuberculous patients has been greatly emphasized in connection with irregular discharge (1–8). However, the adjustment of the patient who remains in the hospital is equally important, since the nature and degree of such adjustment may influence significantly his response to treatment and thereby the course of his illness (9–12).

The kind of adjustment achieved by an individual patient would seem to depend upon two interacting factors: the patient himself, including his personality, adaptive resources, and personal situation; and the particular hospital situation to which he is asked to adjust. In other words, response to hospitalization, like all behavior, is both situationally and characterologically determined.

Most studies of tuberculous patient adjustment have concentrated on the characterologic aspect of the problem, seeking to determine personality characteristics and other patient variables associated with adjustment failure, and using irregular discharge as the criterion of such failure (1–6, 8). An implicit assumption of such studies is that the situational factor may be regarded as constant. The present study, emphasizes, instead, the specific hospital situation to which the patient is asked to adjust. At the same time, an attempt has been made to control to some degree the characterologic factor through differentiated analysis of the patient sample in terms of age, race, and previous hospitalization record.

A hospital, like any other institution, provides for its charges a distinctive psychologic climate or social atmosphere. Such atmosphere, while influenced somewhat by the hospital's location, physical plant, and similar factors, is primarily a reflection of staff behavior and attitudes, and the administrative policies they implement. Wilmer (7) compared tuberculous patients in the two divisions of a large metropolitan sanatorium with respect to various aspects of self-discharge behavior. Whereas physical facilities, food, financial appropriation, et cetera, were identical for the two divisions and admissions were randomly assigned, the divisions were separately staffed and differences in treatment philosophy and administrative policy were readily discernible. Wilmer found very significant differences in self-discharge rates and in other aspects of self-discharge behavior. He concluded that such differences were attributable to distinctive psychologic climates in the two divisions.

The present study extends Wilmer's method to the ward level. The ward, as the basic organizational unit in the hospital, provides an especially meaningful and potentially useful unit of analysis. It is postulated that each ward provides for its patients a specific psychologic atmosphere distinguishable in varying degrees from the atmosphere of any other ward. The psychologic atmosphere of a particular ward may be conceived as a reflection of the attitudes and behavior of all ward personnel (doctor, nurses, and aides) and their interactions with the patients, with each other, and with off-the-ward staff personnel. By virtue of his special role and his position as chief administrator and policy maker on the ward level, the ward physician is ordinarily the most influential single contributor to ward atmosphere.

This study was designed to answer two questions: (*1*) Do tuberculous patients on different wards vary significantly in adjustment to hospitalization, as manifested by irregular discharge rate and length of ward stay? (*2*) Are there significant differences between wards in adjustment to hospitalization of special patient categories: white, non-white, older, younger, first admissions, and re-entries?

Method

Crump and Kennedy, the two Veterans Administration hospitals in Memphis, Tennessee, were ad-

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ministratively consolidated under single management, effective January 9, 1957. The present study included all wards of the consolidated hospital devoted to the active treatment of diagnosed tuberculous patients. (The tuberculosis admission wards and the rehabilitation ward were excluded for greater sample homogeneity.) There were eight such wards at the time of consolidation and throughout the period covered by this study: Wards W, X, Y, and Z of the Crump Division; and Wards A, B, C, and D of the Kennedy Division.

Ward physician assignments were unchanged during the course of this study and other ward staff remained essentially constant. Extensive changes in ward staff assignment and administrative procedure accompanying actual physical consolidation of the two hospitals forced discontinuance of the study after eight months, rather than the full year originally contemplated. The Crump Division was officially closed on April 8, 1958, and its remaining patients and staff were absorbed by the Kennedy hospital.

The sample includes all of the patients discharged from the consolidated hospital during the eight-month period from January 1, 1957, through August 31, 1957, who had spent time on one or more of the tuberculosis treatment wards during the course of hospitalization. For each such patient the following data were compiled: age on admission, race, whether first admission or reentry (at this hospital only), days spent on each ward or wards included in this study, and type of discharge therefrom. The writers are indebted to the Medical Records Library Service for the compilation of these basic data.

In those instances in which a patient spent time on two or more of the wards studied, each ward stay was treated as an independent event and the discharge from each ward was separately enumerated. Medically sanctioned discharges, transfers to other wards, transfers to other hospitals, and deaths were classified as regular discharges. All unsanctioned and disciplinary discharges were classified as irregular discharges.

Results

During the period studied there were 412 discharges from the eight wards involved, 270 (66 per cent) regular and 142 (34 per cent) irregular. Comparison of the irregular discharge rates for Negroes versus whites, older patients (age forty and more) versus younger patients (less than forty), and first admissions versus reentries reveals no statistically significant differences, even at the 10 per cent probability level. This is true for the sample as a whole and for each division considered separately. It is assumed, therefore, that any differences in the distribution of these special patient categories may be disregarded in the interdivisional and inter-ward comparisons which follow.

TABLE 1

Irregular Discharge Rate by Division and Ward

Unit	Regular Discharges	Irregular Discharges	Total Discharges	Per Cent Irregular
Ward A	46	4	50	8
Ward B	36	6	42	14
Ward C	33	8	41	20
Ward D	36	9	45	20
Kennedy Division	151	27	178	15
Ward W	39	37	76	49
Ward X	24	22	46	48
Ward Y	37	26	63	41
Ward Z	19	30	49	61
Crump Division	119	115	234	49
Both Divisions	270	142	412	34

Irregular discharge rates: The distribution of regular and irregular discharges by division and ward is shown in table 1. The difference between irregular discharge rates for the two divisions is highly significant ($chi^2 = 50.18$, $P < 0.001$). Furthermore, the irregular discharge rate for each individual ward at Crump Division significantly exceeded the rate on any Kennedy Division ward.

The significance of inter-ward differences in irregular discharge rate within each division is not statistically demonstrable at the 5 per cent criterion level for ward samples of this size. At Crump Division, however, the difference in rates for Ward Y (41 per cent) and Ward Z (61 per cent) falls just short of statistical significance ($chi^2 = 3.63$, $P < 0.10$. A chi^2 of 3.84 is required for significance at the 5 per cent level).

Special patient categories: The irregular discharge rates for special patient categories by division and ward are seen in table 2. For each category separately considered, as for the patient sample as a whole, the irregular discharge rate at Crump Division significantly exceeded that at Kennedy Division ($P < 0.001$ for each category).

Comparison at the ward level, however, reveals exceptions to the over-all superiority of Kennedy Division. For white patients, the difference in irregular discharge rates between

TABLE 2

PERCENTAGE OF IRREGULAR DISCHARGE RATES FOR SPECIAL PATIENT CATEGORIES BY DIVISION AND WARD

Unit	White	Negro	Younger (Under 40)	Older (40 and Over)	First Admissions	Re-entries	All Patients
Ward A	10	5	5	10	10	7	8
Ward B	18	0	22	8	16	13	14
Ward C	22	11	38	8	33	9	20
Ward D	20	30	17	23	11	27	20
Kennedy Division	17	10	20	12	17	14	15
Ward W	52	38	46	51	53	46	49
Ward X	50	43	48	47	53	44	48
Ward Y	35	55	50	34	38	43	41
Ward Z	64	43	50	70	50	68	61
Crump Division	50	46	48	50	49	49	49
Both Divisions	37	28	37	32	34	35	34
Number of patients	303	109	194	218	164	248	412

Crump Ward Y (35 per cent) and Kennedy Ward C (22 per cent) is statistically insignificant and could have occurred easily by chance. For Negro patients, the difference in irregular discharge rates between Crump Ward W (38 per cent) and Kennedy Ward D (30 per cent) is likewise statistically insignificant.

For younger patients (less than forty) the irregular discharge rate of 38 per cent on Kennedy Ward C was not significantly different from the corresponding rate of 48 per cent for the Crump Division as a whole, nor did it differ significantly from the rate for any individual ward within the Crump Division. For older patients (forty and more), the irregular discharge rate on Kennedy Ward D (23 per cent) was significantly lower than the corresponding rate of 70 per cent on Crump Ward Z ($chi^2 = 9.15$, $P < 0.01$), but did not differ significantly from the corresponding rates of 34 and 47 per cent on Crump Wards Y and X, respectively. The difference in irregular discharge rates for older patients on Kennedy Ward D (23 per cent) and Crump Ward W (51 per cent) fell just short of statistical significance at the criterion level ($chi^2 = 3.49$, $P < 0.10$. A chi^2 of 3.84 is required for significance at the 5 per cent level).

For first admissions, the irregular discharge rate of 33 per cent on Kennedy Ward C did not differ significantly from the rates for first admissions on any of the individual Crump wards. For re-entries, the irregular discharge rate of 27 per cent on Kennedy Ward D did not differ significantly from the rates for re-entries on Crump Wards W, X, and Y, but was significantly lower than the 68 per cent on Crump Ward Z ($chi^2 = 7.84$, $P < 0.01$).

To summarize: (*1*) the over-all irregular discharge rate for Crump Division significantly exceeded that for Kennedy Division; (*2*) the over-all irregular discharge rate for each individual ward at Crump Division significantly exceeded the rate on any Kennedy Division ward; (*3*) the irregular discharge rate of Crump Division for every special patient category significantly exceeded the corresponding rate at Kennedy Division; (*4*) comparisons of individual Crump Division wards with individual Kennedy Division wards with respect to irregular discharge rates for special patient categories revealed for each such category exceptions to the superiority consistently shown by Kennedy Division in interdivisional comparisons. It should also be noted that no single Crump or Kennedy ward was responsible for these exceptions, but that rates for different patient categories varied considerably on the same ward.

Within the Crump Division the irregular discharge rate for white patients on Ward Z (64 per cent) significantly exceeded the corresponding rate of 35 per cent on Ward Y ($chi^2 = 6.21$, $P < 0.02$). Among older patients the irregular discharge rate of 70 per cent on Ward Z significantly exceeded the corresponding rate of 34 per cent on Ward Y ($chi^2 = 6.56$, $P < 0.02$). For re-entries, the difference between the 68 per cent irregular discharge rate on Ward Z and the 43 per cent rate on Ward Y fell just short of statistical significance at the criterion level ($chi^2 = 3.46$, $P < 0.10$. A chi^2 of 3.84 is required for significance at the 5 per cent level).

Significance of inter-ward rate differences for other patient categories among Crump wards were not statistically demonstrable. Within the Kennedy Division the ward rates for white patients did not differ significantly, and ward samples for other patient categories were too small for statistical comparison. However, the significant differences found between Crump

TABLE 3

PERCENTAGE OF TYPES OF REGULAR DISCHARGE BY DIVISION AND WARD

Unit	Maximal Hospital Benefit	Transfer to Another Ward	Hospital Transfer or Death
Ward A	50	43	7
Ward B	44	48	8
Ward C	36	52	12
Ward D	17	72	11
Kennedy Division	38	53	9
Ward W	44	44	12
Ward X	67	29	4
Ward Y	70	14	16
Ward Z	53	37	10
Crump Division	58	30	12
Both Divisions	47	43	10
Number of patients	126	116	28

Division wards, despite limited samples, sufficed to demonstrate that the irregular discharge rates for special patient categories varied significantly from ward to ward, even within the same division.

Types of regular discharge: In table 3, the regular discharges for division and ward are classified according to type. The table indicates wide variation in the relative proportion of the two principal types, medically sanctioned discharges and inter-ward transfers, which tend to vary inversely with one another.

Fifty-three per cent of the regular discharges at Kennedy Division were inter-ward transfers, as compared with 30 per cent at Crump Division. The difference is highly significant ($chi^2 = 10.15$, $P < 0.01$), and raises the question of whether the more frequent patient "shuffling" at Kennedy Division may have contributed in some way to the much lower irregular discharge rate at this division.

Ward physicians are sometimes suspected by other ward physicians of selecting for transfer their "problem cases," patients whose histories and ward behavior suggest greater probability of irregular discharge. To determine if such a selective factor was operating in this study, the final dispositions of all inter-ward transfers within divisions and between divisions was ascertained, disregarding 28 transfers to surgical and rehabilitation wards not included in this study.

The majority of inter-ward transfers from Kennedy wards were to other Kennedy treatment wards (47 patients) rather than to Crump treatment wards (26 patients). Only 6 patients were transferred from Crump wards to Kennedy treatment wards, and only 9 patients to other treatment wards within the Crump Division. Irregular discharge rates for the patients transferred, both between divisions and within divisions, did not differ significantly from the prevailing rates at the respective divisions. These findings clearly exclude selective patient transfers as a significant factor in the irregular discharge rates obtained at the two divisions.

For individual wards the proportion of medically sanctioned discharges ranged from 17 to 70 per cent, and the proportion of inter-ward transfers, from 14 to 72 per cent, with the significance of the differences statistically demonstrable in most instances. Rank order correlation between the proportion of inter-ward transfers and irregular discharge rates for the eight treatment wards revealed no significant relationship (Rho = 0.423. A coefficient of 0.643 is required for significance at the 5 per cent level). This finding indicates that the irregular discharge rate of the transferring ward was not significantly affected by the proportion of its patients transferred to other wards. The beneficial effects of inter-ward transferring, suggested by the earlier interdivisional comparison, seem to be reflected primarily in the irregular discharge rates of the receiving wards, as would be expected on *a priori* grounds.

Types of irregular discharge: Wilmer (7) has shown the importance of distinguishing between the different types of irregular discharge, since each type may present distinctive psychologic implications.

In the medically unsanctioned type of discharge (signed release, against medical advice), the ward physician is confronted directly with the patient's problem or alleged problem, and afforded an opportunity to deal with it. In the other type (failure to return from authorized leave), no such opportunity is presented, and the patient's behavior may be determined primarily by circumstances he encounters outside the hospital. Disciplinary discharge ordinarily reflects direct defiance by the patient of rules and regulations variably interpreted, explained, and enforced on the different wards.

TABLE 4
PERCENTAGE OF TYPES OF IRREGULAR DISCHARGE

	Medically Unsanctioned	Failure to Return from Leave	Disciplinary
Kennedy Division	22	70.5	7.5
Crump Division	46	45	9
Both Divisions	42	50	8
Number of patients	59	71	12

TABLE 5
WARD STAY: PERCENTAGE OF REGULAR DISCHARGES

Unit	Less Than One Month	One Month or More
Ward A	26	74
Ward B	25	75
Ward C	30	70
Ward D	28	72
Kennedy Division	27	73
Ward W	51	49
Ward X	46	54
Ward Y	35	65
Ward Z	63	37
Crump Division	47	53
Both Divisions	36	64
Number of patients	97	173

A breakdown of these three types of irregular discharge from each division is given in table 4. At Kennedy Division, 22 per cent of the irregular discharges were medically unsanctioned and 70.5 per cent were failures to return from leave, as compared with 46 per cent and 45 per cent for the corresponding discharges at Crump Division. The difference is statistically significant ($chi^2 = 4.69$, $P < 0.05$).

Ward stay: Regular versus irregular discharge has been used in this study as the principal criterion of hospital adjustment. Length of stay on the discharging ward merits consideration as a supplementary criterion.

The percentage of regularly discharged patients who remained on the discharging ward less than one month and the percentage who stayed a month or longer are shown by division and ward in table 5. At Kennedy Division, 27 per cent of the regular dischargees stayed less than a month, as compared with 47 per cent at Crump Division. The difference is highly significant ($chi^2 = 10.84$, $P < 0.001$). Thus the regular discharges at Kennedy Division, on the whole, represent longer periods of ward adjustment than those at Crump Division.

Comparisons of irregular discharge rates for the various wards (*see* table 1) and the percentage of regular dischargees staying less than one month (*see* table 5) reveal a very strong relationship. Rank order correlation of these two variables confirms the highly significant relationship between these two independent criteria of ward adjustment ($Rho = 0.97$, $P < 0.01$).

In table 6 a similar breakdown of the irregularly discharged patients is given, showing by division and ward the percentage who stayed on the discharging ward less than three months and the percentage who stayed three months or longer. At Kennedy Division, 67 per cent of the irregular dischargees left the hospital in less than three months, as compared with 48 per cent at Crump Division. While the difference falls short of statistical significance at the criterion level, it is interesting to note that the ward stay pattern for irregular discharges at the two divisions is the converse of that for regular discharges. At Kennedy Division, where a much lower irregular discharge rate prevailed, the regular dischargees generally stayed longer than at Crump Division, while the irregular dischargees tended to leave earlier.

For individual wards, the percentage of pa-

TABLE 6
WARD STAY: PERCENTAGE OF IRREGULAR DISCHARGES

Unit	Less than Three Months	Three Months or More
Ward A	75	25
Ward B	83	17
Ward C	62	38
Ward D	56	44
Kennedy Division	57	43
Ward W	59	41
Ward X	55	45
Ward Y	38	62
Ward Z	37	63
Crump Division	48	52
Both Divisions	51	49
Number of patients	73	69

TABLE 7

INTER-WARD COMPARISON OF DISCHARGE RATES FOR A RESTRICTED CATEGORY: THE OLDER WHITE PATIENT

	Ward Y	Ward Z
Regular	19 (79%)	8 (31%)
Irregular	5 (21%)	18 (69%)
Total	24 (100%)	26 (100%)

$Chi^2 = 9.90$, $P < 0.005$.

tients irregularly discharged after a stay of three months or more and the irregular discharge rate are significantly related, as demonstrated by rank order correlation of these two variables (Rho = 0.79, $P < 0.05$). In other words, the irregular discharges tended to occur after longer ward stay on wards with a higher irregular discharge rate.

Restricted patient categories: The preceding analysis has shown that irregular discharge rates for special patient categories (Negro versus white, older versus younger, and first admission versus re-entry) may vary significantly from ward to ward when there are no significant differences for the patient sample as a whole. The size of the sample limits inter-ward comparisons for more restricted patient categories, but one such category, the older white patient, has been selected to illustrate the potentialities of further differentiation.

The discharge rates for the older white patient on two wards of the same division are compared in table 7. The striking difference in irregular discharge rates could have occurred by chance less than five times in a thousand ($chi^2 = 9.90$, $P < 0.005$). As shown earlier, comparison of rates on the same two wards for the separate categories, white patients and older patients, yielded probability values considerably smaller ($P < 0.02$ in each case).

GENERAL OBSERVATIONS

At the time of administrative consolidation, when this study began, the Kennedy Division was a 1,250-bed general hospital embracing the highest types of almost all fields of medical and surgical practice. The Crump Division was a specialized tuberculosis sanatorium type of hospital of 300 beds. At Kennedy, the tuberculosis physicians were younger men, actively interested in and profoundly influenced by the associated specialized fields with which they were in almost daily contact. At Crump the medical staff consisted of older physicians, often with many years' experience in the treatment of tuberculosis, but more or less isolated from the general practice of medicine. Since the physician assignments to the wards studied remained constant throughout the period covered, this over-all distinction between the two divisions was perpetuated.

The two divisions also differed somewhat in general admission policy, particularly so during the pre-consolidation era, when a substantial portion of the patients involved in this study entered the hospital. The Crump Division, dependent upon tuberculous patients alone to maintain the hospital's operating capacity, was more inclined to readmit former patients with previous records of poor hospital adjustment and patients whose tuberculosis was complicated by alcoholism or other socio-medical disorders.

One difference in operating procedure at the two divisions is worthy of note. At Kennedy the ward physician took charge of his patients from the moment of admission to the hospital, obtained their histories, and personally performed the complete physical examinations. At Crump the established practice was to have the initial examinations performed by a special admitting physician, who made the diagnosis and then transferred the patients to their ultimate ward physicians for treatment. Although the Crump procedure was changed after consolidation to conform with the Kennedy practice, many of the Crump Division patients included in this study had been processed in the manner described when admitted.

It should also be noted that the wards at Kennedy were considerably smaller than those at Crump, and the Crump physicians had larger patient loads than those at Kennedy. The Crump physician carried, on an average, 42 patients on a single 49-bed capacity ward. The Kennedy physician was responsible for an average of only 34 patients, split between two wards: a tuberculosis treatment ward with an average capacity of 27 beds and the admission-diagnostic ward where 9 beds were assigned him. Within each division the operating capacity and patient census of individual wards remained essentially uniform throughout the period studied.

Some differences between the two divisions would seem to favor Crump over Kennedy.

Crump offered its tuberculous patients their own chapel, a complete library, and beautiful grounds over which all noncontagious patients could wander almost at will. Television sets were available at a rate of about one for every 3 or 4 patients. A central radio broadcasting system, with individual earphones, made possible quiet listening in bed for every patient. The resources of the entire hospital, including volunteer units, were directed toward making the tuberculous patient's hospital stay as enjoyable as possible in a friendly, paternalistic atmosphere.

For tuberculous patients at Kennedy there was no separate library or chapel, use of the hospital grounds was restricted, and only two television sets per 28-bed ward were available. There was no central radio broadcast, and the bedside table radios provided were used at times without consideration for nearby patients otherwise engaged. While the hospital did its best to make the stay pleasant for its 200 tuberculous patients, its major effort, naturally, was in behalf of the general medical and surgical patients who formed the majority of its bed occupants.

Fundamental differences in treatment philosophy at the two divisions, while not readily amenable to statistical analysis, were clearly apparent in distinctive practices, formalized policies, and staff behavior. At Crump the patient's social welfare and economic situation were heavily emphasized, systematically evaluated, and dealt with through an elaborate, highly organized rehabilitation program and comprehensive Social Service coverage, a factor Tollen (6) has stressed as a preventive of irregular discharge. Hospitalization was often prolonged as much as six months in an attempt to improve the patient's employability and socio-economic status.

On the other hand, the patient was rarely given any specific information about the status or prognosis of his disease, and the vague and incomplete information he might occasionally elicit was often given summarily and at the patient's insistence. Major decisions as to treatment were made by the Chief of Service and relayed to the patients by the ward physician with varying degrees of effectiveness. Although a change in these policies was made after consolidation of the two hospitals so as to conform with the Kennedy practice, some ward physicians found it difficult to modify such long-established attitudes.

At Kennedy, staff efforts were concentrated on a single objective: to get the patient well and out of the hospital as early as his medical condition would permit. Socio-economic factors were not systematically singled out for special attention in every case as at Crump, and such factors were not generally permitted to prolong the hospital stay to any significant extent. Social Service coverage was barely adequate and the rehabilitation program was rudimentary and loosely organized.

However, the patient at Kennedy was constantly and fully informed of his medical status, the course of his illness and prognosis, and his understanding and cooperation were actively solicited. He was told not only what had to be done, but why, and what he could do to facilitate recovery. Such practices were formally delineated and actively promoted by the Chief of Service as standard policy.

Discussion

Most investigators of the irregular discharge problem among tuberculous patients have reported a greater probability of irregular discharge for patients with a history of irregular discharge (1, 3–6, 8). Unfortunately, the data available for this study did not include the type of discharge received on previous admissions. An attempt was made to control this factor indirectly through classification of patients as first admissions and re-entries (at this hospital only), and no significant difference was found in the irregular discharge rate for these two categories, or in the relative proportions of first admissions and re-entries for the two divisions. However, the facts that previous hospitalizations at other hospitals were not considered in this classification and that a less discriminating admission policy apparently prevailed at Crump Division may have introduced a selective factor which was responsible to some unknown degree for the conspicuous interdivisional differences in patient adjustment.

The variable results of interdivisional comparisons for special patient categories, and the significant differences in patient adjustment among wards of the same division, cannot be accounted for in terms of differential admission policy. Within each division, patients were as-

signed to the various wards on a random basis, and there is no reason to assume other than random distribution with respect to irregular discharge history or other unfavorable characteristics.

Curtailment of the sample by premature termination of the study reduced considerably the likelihood of demonstrably significant inter-ward differences, particularly with respect to special patient categories. The consistent trend of the findings despite this limitation suggests that other differences noted would have been raised to statistical significance by larger samples.

It is true that the period studied was a trying one for the Crump Division. The administrative consolidation with Kennedy in January, 1957, stimulated continuous rumors of the imminent closure of the Crump Division, resulting in noticeable deterioration of morale among employees who perceived in such closure a threat to their jobs or job status. Inevitably, some of their anxiety and pessimism seeped through to the patients at times. However, the irregular discharge rate at Crump Division for the entire year of 1957 (44 per cent) was exactly the same as the rate for 1956, and slightly lower than for 1955 (47 per cent), suggesting that lowered employee morale associated with the consolidation was not a major factor in the findings.

Although the wards at Kennedy were considerably smaller physically than those at Crump, the effect of this factor in itself is difficult to evaluate. Within each division the operating capacity and patient census of the wards remained fairly uniform throughout the period studied, yet inter-ward differences in irregular discharge rates were frequently quite marked both for patients in general and for special patient categories.

Some patients may have felt the social stigma of their disease more keenly at Crump because it was known to be a tuberculosis hospital, thus making their diagnosis apparent to their townsfolk. Such patients may have preferred Kennedy merely because it was a general hospital. Tolen (6) found the irregular discharge rate to average 13 per cent less in general hospitals than in tuberculosis hospitals.

A patient admitted to Kennedy was apt to obtain the impression of a highly competent, highly efficient organization capable of dealing successfully with every treatment problem he might present. A patient admitted to Crump encountered a closer knit but less efficient social organization, and a medical staff relying heavily on outside consultants for any treatment problems other than tuberculosis itself. Thus the Kennedy Division would appear to have been more conducive to patient feelings of confidence in its medical staff than was the Crump Division.

The most significant difference between the two divisions appears to have been one of prevailing treatment philosophy and the deeply ingrained staff attitudes and practices associated therewith. The Crump orientation could be characterized as benevolent, paternalistic, and autocratic. Access to detailed information about the patient's condition and treatment was zealously guarded as primarily a medical prerogative. While social and economic factors were officially emphasized and routinely dealt with, psychologic factors were minimized or ignored. The established intake procedure, divorcing the diagnostic and treatment functions, left room for doubt in the patient's mind that the doctor who was treating him fully understood his personal situation and the nature and extent of his illness.

At Kennedy, the role of the ward physician was much more demanding. His primary responsibility for total patient care was clearly established, and the patient–doctor relationship was officially recognized and stressed as the keystone of effective treatment. The fact that he personally handled the initial history and physical examination laid the groundwork for an effective patient–doctor relationship, and helped to assure the patient that his doctor fully understood both his illness and his personal situation. The close and continuing relationship was calculated to assure the patient that his illness was thoroughly understood and its course carefully followed, to instill in him a sense of personal responsibility for and active participation in his recovery, and to share with him the satisfaction of observable progress toward the mutual goal.

The marked interdivisional difference in irregular discharge rates suggests that most patients appreciated time-conscious, efficient treatment of their disease more than they did intensive efforts to improve their socio-economic status. It suggests also that the majority of patients respond favorably to the concept of treatment as a cooperative enterprise of patient and physician in which the patient role is one of active participant rather than passive recipient.

The inter-ward differences in patient adjust-

ment found at each division probably reflect the various abilities of the respective ward physicians and their capacities to form and maintain effective patient–doctor relationships. The physician with the highest irregular discharge rate at Crump would refer to his patient on medical rounds, in the patient's presence, as "this consumptive," and in other ways suggested lack of personal interest in his patients. In contrast, the Crump physician with the lowest rate clearly demonstrated his deep feeling for his patients as people by his concern for their personal, financial, and social circumstances, as well as for their disease. The practice of assigning physicians of lesser qualifications and ability to tuberculous patients is not uncommon, and the results such physicians obtain are usually commensurate with what they would accomplish in other less specialized fields. Success in treating long-term illnesses, such as tuberculosis, seems to depend considerably on the physician's effectiveness as a person as well as on his technical competence (7, 10–12).

One of the findings suggests that transferring patients from one ward to another may in itself contribute to hospital adjustment. There was no difference in intention at the two divisions in this regard, and this finding came as a complete surprise. One can only speculate that such transfers were perceived by the patients involved as welcome diversions, bringing transient relief from monotonous surroundings and boring routine.

At Kennedy, with its much lower irregular discharge rate, the irregular discharges tended to occur earlier than at Crump, while the regularly discharged patients stayed longer. At Crump the converse pattern was found. The patient who leaves the hospital irregularly after only a brief stay is much more likely to have basic personality deficiencies which preclude good adjustment to almost any situation. On the other hand, the patient who leaves irregularly after a relatively long stay has demonstrated by such a stay some capacity for hospital adjustment; his irregular discharge is more likely to be a reaction to the ward situation itself. Thus the briefer stay of irregular dischargees at Kennedy suggests that such discharges were more often characterologically determined, whereas those at Crump were more often situationally determined. This interpretation is consistent with the general findings and militates against differential admission policy as a major factor in the interdivisional differences found.

Summary

An analysis was made of the 412 discharges occurring over an eight-month period from eight tuberculosis treatment wards, divided equally between the two divisions of a newly consolidated Veterans Administration hospital. Divisions and wards were compared with respect to hospital adjustment of special patient categories, as well as patients in general. Adjustment was defined primarily in terms of regular versus irregular discharge; the length of ward stay was investigated as a supplementary criterion.

The results of the study support the following conclusions:

Patient adjustment to hospitalization may vary significantly from ward to ward of the same hospital.

Special patient categories (Negro, white, older, younger, first admission, re-entry) may adjust differently on different wards independently of the adjustment level for patients in general.

Patient adjustment may be improved by selective ward assignment, based on established differences in effectiveness with special patient categories.

More frequent inter-ward transfer may improve hospital adjustment by varying the stimulation to which patients are exposed, thereby reducing monotony and "hospital fatigue."

A more direct and promising approach to the problem depends upon positive identification of factors responsible for the inter-ward differences demonstrated in this study.

The method and conclusions of this study of hospitalized tuberculous patients may be equally applicable to patients suffering from other chronic illnesses requiring prolonged hospitalization.

Sumario

El Ajuste Hospitalario de los Tuberculosos en Distintas Salas del Mismo Hospital

Este análisis versa sobre las 412 altas que tuvieron lugar durante un período de ocho meses en ocho salas dedicadas al tratamiento de la tuberculosis, divididas por igual entre las dos divisiones de un nuevo hospital consolidado de la Administración de Veteranos. Se compararon las divisiones y las salas con respecto al ajuste hospitalario de ciertas clases de enfermos, así como de los enfermos en general. El ajuste fué definido primordialmente en términos de alta regular o irregular; como pauta complementaria se investigó la duración de la permanencia en la sala.

Los resultados del estudio apoyan las siguientes conclusiones:

El ajuste de los enfermos a la hospitalización puede variar notablemente de sala a sala en el mismo hospital.

Varias clases de enfermos (negros, blancos, más viejos, más jóvenes, primer ingreso, reingresados) pueden ajustarse de modo distinto en diversas salas, independientemente del tenor del ajuste para los enfermos en general.

Cabe mejorar el ajuste de los enfermos mediante la distribución selectiva a las salas, basado esto en las diferencias notadas en eficacia con respecto a ciertas clases de enfermos.

Los traslados más frecuentes entre salas pueden mejorar el ajuste al hospital por variar el estímulo que reciben los enfermos, reduciendo así la monotonía y la "fatiga hospitalaria."

La obtención de una vía más directa y fértil al problema se basa en la identificación positiva de los factores que ocasionan las diferencias entre salas, observadas en este estudio.

El método seguido en este estudio de los tuberculosos hospitalizados y las conclusiones alcanzadas pueden tener aplicación igual a los enfermos que padecen de otras dolencias crónicas que requieren hospitalización prolongada.

Resume

Adaptation à la vie hospitalière des tuberculeux hospitalisés dans différentes salles d'un même hôpital

Analyse de 412 "sorties d'hôpital" pendant une période de huit mois. Ces cas ayant été hospitalisés dans huit différentes salles d'hôpital réparties également entre les deux divisions réunies d'un nouvel hôpital de *Veterans Administration*. On a comparé les diverses divisions et salles sur le plan de l'adaptation à l'hôpital de catégories spéciales de malades et des malades en général. L'adaptation était d'abord définie en termes de sortie régulière ou de sortie irrégulière (sans approbation médicale); l'investigation de la durée du séjour à l'hôpital constituait un critère supplémentaire.

Les résultats de l'étude confirment les conclusions suivantes:

L'adaptation du malade à l'hospitalisation peut varier de façon significative d'une salle à l'autre dans un même hôpital.

Des catégories spéciales de malades (nègres, blancs, malades plus âgés ou plus jeunes, première admission à l'hôpital, nouvelle admission) peuvent s'adapter différemment selon les différentes salles indépendamment des malades en général.

L'adaptation du malade peut être améliorée en lui assignant une salle sélectionnée, en se fondant sur les différences et d'efficacité établies selon la catégorie spéciale à laquelle appartient le malade.

De plus fréquents transferts d'une salle à l'autre sont susceptibles d'améliorer l'adaptation à la vie hospitalière grâce à la variation des conditions de stimulation auxquelles les malades sont exposés, d'où réduction de la monotonie et de la "fatigue hospitalière."

La possibilité d'aborder ce problème plus directement et de façon plus encourageante dépend de l'identification positive des facteurs responsables des différences existant entre les salles, qui sont démontrées dans cette étude.

Les méthodes et les conclusions de cette étude sur le malade tuberculeux hospitalisé sont également applicables aux malades affectés d'autres maladies chroniques exigeant l'hospitalisation prolongée.

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PURIFIED PROTEIN DERIVATIVES (PPD) AND OTHER ANTIGENS PREPARED FROM ATYPICAL ACID-FAST BACILLI AND NOCARDIA ASTEROIDES[1, 2, 3, 4]

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Introduction

There have been many reports in the literature of atypical acid-fast bacilli which have been associated with human infection. That these organisms are atypical mycobacterial strains which are the etiologic agents of human pulmonary disease has been considered by many investigators (1–4). Moreover, the possibility that these organisms may cause nonspecific tuberculin sensitivity in man has recently been reported by Edwards and Palmer (5) and has led to provocative implications. These current reports have stimulated much interest in the relationship of the atypical acid-fast bacilli to tuberculosis and tuberculosis-like disease and have emphasized the need for a systematic study of this group of organisms.

Since there is little information on the chemistry of these atypical strains, investigations of this nature would help not only to define more clearly this group of organisms but also to establish their clinical significance. Therefore, four strains of atypical acid-fast bacilli and one strain of *Nocardia asteroides*, all isolated from human infections, were grown on liquid medium. Purified protein derivatives (PPD) and other antigens were prepared from them, and the chemical and biologic properties of these preparations were studied. The latter included sensitizing of laboratory animals and testing their cross-immunologic reactions by skin tests, precipitin tests, and agar gel technique.

[1] From The Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Based, in part, on thesis material submitted to the Faculty of the Graduate School of Duke University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Presented, in part, before the Medical Session, as part of *Section 4B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 20, 1958.

[4] Aided by assistance from a cooperative project with the Division of Special Health Services, U. S. Public Health Service.

Materials and Methods

Sources of organisms: Four strains of atypical acid-fast bacilli and one strain of *Nocardia asteroides* used in this study were obtained from the following sources:

Kansas City I and Kansas City II strains (Bostrom D-35 and Forbes-84, respectively) were isolated by Pollak and Buhler (1) in Kansas City from patients with disease resembling tuberculosis. These are referred to as the yellow bacillus by these writers and are considered photochromogens according to the classification of Runyon (6).

Battey I and Battey II strains (Battey No. 100616 and Battey No. 121326, respectively) were isolated from 2 patients at the Battey State Hospital, Rome, Georgia, and are representative of the organisms described by Crow and associates (7). They were generously supplied by Dr. C. Edwin Smith.

The *Nocardia asteroides* strain (No. 2412) was isolated from the sputum and a metastatic lesion of the leg of a patient at Duke University Medical Center and kindly given by Dr. David T. Smith and Dr. Norman F. Conant.

These cultures were grown on tubes of solid glycerol agar and Long synthetic medium and were routinely transplanted every four to six weeks. The organisms were also seeded on Long synthetic liquid medium and, when sufficiently grown, this seed was used as the inoculum for the growth of the organisms to be used as the source of the PPD.

Preparation of the PPD: The method used for the preparation of the PPD was according to that of Seibert (8) for preparing the international standard tuberculin PPD-S, which consisted briefly of autoclaving of the eight- to ten-week-old cultures, concentration by ultrafiltration at 4°C. of the bacteriologically filtered culture filtrate, and precipitation of the protein (PPD) by neutral ammonium sulfate at half saturation.

It should be pointed out that new glassware and apparatus were used in the preparation of each of these PPD preparations in order to eliminate contamination which might cause cross-reactions.

Methods of Chemical Analysis

Nitrogen determinations: Total nitrogen was determined by the micro-Kjeldahl nesslerization procedure of Miller and Miller (9), modified at a level of 10γ of nitrogen. The protein was calculated from this by the use of the factor 6.25.

Nucleic acid: Nucleic acid, calculated as desoxyribonucleic acid, was determined by a modification

of the Dische diphenylamine method and adopted to quantitative analysis by Seibert (10). Increased sensitivity in the latter method was obtained by incubation at 37°C. instead of boiling for fifteen minutes, according to the suggestion of Patterson and Dackerman (11). Ribonucleic acid, was determined by the orcinol reaction of Drury (12), modified by Miller, Goldner, and Miller (13).

Carbohydrate: Carbohydrate, calculated as glucose, was determined by the Koehler modification (14) of the Morris anthrone method, using a ten-minute heating period.

Ultraviolet spectral absorptions: Ultraviolet spectral absorptions were measured in the Beckman spectrophotometer, model DU.

Immunologic Methods

Antisera: Sera containing antibodies to these organisms and also to the BCG strain were obtained from sensitized commercially available male rabbits weighing from 2,200 to 3,200 gm. In order to induce antibodies, the rabbits had been injected with 10 to 15 doses of the antigen prepared from the organisms as described below. The rabbits were bled at intervals from a slit in the marginal ear vein, and the serum was separated and stored at −6°C.

Preparation of antigens for rabbit sensitization: Cultures of each strain of organisms, which had been grown on Long liquid medium, were autoclaved for thirty minutes at 15 pounds of pressure. The bacillary suspensions were then centrifuged for one hour, the supernatants decanted, and the packed organisms spread out against the side of the centrifuge tubes. The tubes were placed in a desiccator and dried in vacuo at 37°C. The dried bacillary bodies were ground in a mortar during the addition of sterile, light mineral oil until a fine suspension was obtained. The rabbits were injected at weekly intervals on alternate sides with 0.1 ml. of the suspensions containing 0.4 mg. of the organisms.

Preparation of antigens for guinea pig sensitization: Guinea pigs weighing more than 500 gm. were sensitized with either the human Jamaica 22 strain or the human DT strain suspended in oil, according to the method of Freund and Gottshall (15). At the end of six weeks the animals were tested for sensitivity with PPD-S. Only those guinea pigs which had attained good sensitivity to PPD-S were used for subsequent skin tests.

Precipitin tests: The method described by Seibert and associates (16) was used. Antigens were prepared from each of the PPD preparations by dissolving them in 0.9 per cent saline containing 0.01 per cent merthiolate and were made to the following concentrations on the basis of their protein content: 50γ, 10γ, 5γ, and 1γ. The A protein antigen (1071I) which had been isolated from the raw, unheated culture filtrate of the H37Ra strain grown on Wong-Weinzirl medium (17) was also diluted to the four concentrations as noted above.

Agar-gel technique: The method of Oakley and Fulthorpe (18) of double diffusion, described by Harris and co-workers (19) and modified by Seibert and Soto-Figueroa (20), was used. The antigens used were diluted with buffered saline containing 0.01 per cent merthiolate. The final concentration of the protein antigens was 3 mg. per ml. The tubes were initially read after three, four, and five days, but it was found that after ten days the maximal number of bands had formed. The sharpness and the distance of the bands in millimeters from the serum-agar interface were also noted.

Skin tests in rabbits: Rabbits, which had been previously sensitized to each of the atypical organisms and the *Nocardia asteroides* were skin tested with 0.05 mg. per 0.1 ml. of the homologous PPD and also with PPD-S. Two rabbits which had been sensitized previously with the BCG strain were skin-tested simultaneously with each of the PPD preparations and PPD-S in the same dosage. The reactions were read after twenty-four and forty-eight hours and were measured as two diameters at right angles to each other, and the height of the reactions was estimated on a scale ranging from 1 to 4.

Skin tests in guinea pigs: Twelve guinea pigs which had been previously sensitized with human tubercle bacilli were skin tested with each of the PPD preparations. They were injected with 0.1 ml. containing 0.005 mg. of these protein fractions as well as with PPD-S. The sites of injection were randomized so that the reader did not know their identity. The reactions were read after twenty-four hours in the manner previously described.

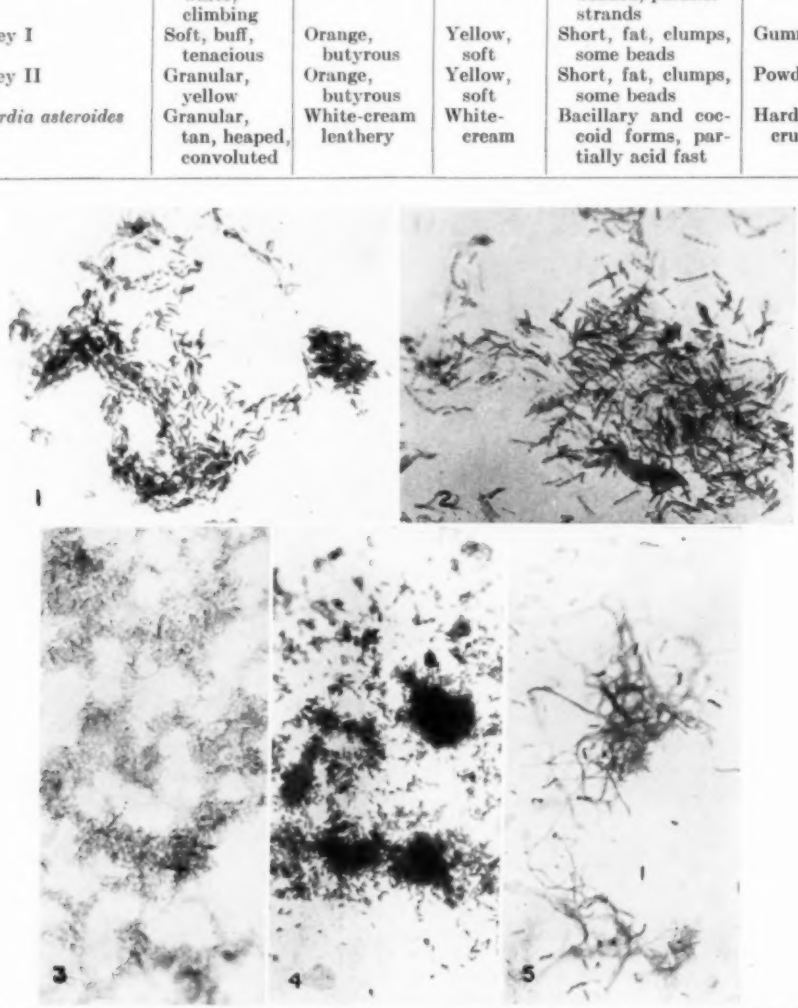
Results

Cultural appearance: A summary of the bacteriologic characteristics of each of the strains from which the PPD preparations were made is given in table 1. Cultural differences appeared when these organisms were grown on Long liquid medium. For example, the two Kansas City strains gave soft, creamy growths which were cream to white in color; but that of the Kansas City I strain was very convoluted, while that of the Kansas City II strain was smooth. The growth of the Battey I strain was soft, tenacious, and buff in color; but it became yellow and convoluted as it aged. In contrast, the Battey II strain, a very slow grower, had a yellow granular pellicle which became orange with age. The growth characteristics of *Nocardia asteroides* were very different from both the Kansas City and Battey strains. It grew in a granular pellicle which later became heaped and deeply convoluted, a characteristic type of growth for this organism. Its color was at first white which later turned to tan.

TABLE 1

BACTERIOLOGIC CHARACTERISTICS OF ATYPICAL ACID-FAST BACILLI

Strains	Cultural Appearance			Microscopic Appearance	Consistency of Dried Bacillary Bodies
	Liquid Long	Solid			
		Long	Glycerol		
Kansas City I	Soft, creamy, convoluted	Buff, soft	Buff, soft	Long, slender beaded, parallel strands	Gummy
Kansas City II	Soft, creamy, white, climbing	Buff, soft	Buff, soft	Long, slender beaded, parallel strands	Gummy
Battey I	Soft, buff, tenacious	Orange, butyrous	Yellow, soft	Short, fat, clumps, some beads	Gummy
Battey II	Granular, yellow	Orange, butyrous	Yellow, soft	Short, fat, clumps, some beads	Powdery
Nocardia asteroides	Granular, tan, heaped, convoluted	White-cream leathery	White-cream	Bacillary and coccoid forms, partially acid fast	Hard and crumbly



FIGS. 1–5. Smears of atypical acid-fast bacilli grown on Long liquid medium and stained by Ziehl-Neelsen method (× 1125). FIG. 1. Kansas City I; FIG. 2. Kansas City II; FIG. 3. Battey I; FIG. 4. Battey II; FIG. 5. *Nocardia asteroides*.

On solid media, all strains except *Nocardia asteroides* initially gave an orange, soft growth on Long medium, but a yellow soft growth on glycerol agar. However, after repeated transfers, the Kansas City strains assumed a cream to buff color on both media while the Battey strains remained essentially unchanged.

That the consistency of the dried bacillary bodies of these organisms differed may be noted in table 1. It was gummy for the Kansas City strains and for the Battey I strain, powdery for the Battey II strain, and hard and crumbly for *Nocardia asteroides*.

Microscopic appearance: Microscopic studies of smears by the Ziehl-Neelsen stain showed that the Kansas City strains were long, slender, beaded, acid-fast rods adhering in parallel strains (figures 1 and 2). In contast, the Battey strains were short, fat, coccoid-like, acid-fast rods which were arranged in clumps (figures 3 and 4). All of these strains were strongly acid fast except *Nocardia asteroides* which exhibited partially acid-fast, branching hyphae together with some bacillary and coccoid forms (figure 5).

Analyses of PPD preparations: Chemical analyses were made on each of the PPD preparations to determine their protein, carbohydrate, and nucleic acid contents. The protein content was estimated on the basis of the true nitrogen values and the percentage of total solids, as protein for the Kansas City strains ranged from 93.5 per cent to 97.7 per cent, while the PPD preparations from the two Battey strains gave intermediate values of 94.2 per cent and 96.8 per cent. Of the total solids present in the Nocardia PPD, 99.1 per cent was found to be protein (table 2).

A marked difference was evident in the carbohydrate content of the PPD preparations from the two Kansas City strains. The PPD from the Kansas City I strain contained four times as much carbohydrate (calculated as glucose) as the PPD from the Kansas City II strain. However, the carbohydrate in the PPD preparations from the two Battey strains did not show much variation (2.1 per cent and 3.7 per cent). The Nocardia PPD contained the least amount of carbohydrate, only 0.8 per cent (table 2).

There was also a marked difference in the percentage of desoxyribonucleic acid, determined by the Dische diphenylamine reaction, using thy-

TABLE 2

CHEMICAL ANALYSES OF THE PPD PREPARATIONS FROM ATYPICAL ACID-FAST BACILLI

PPD	Per Cent of Total Solids as		
	Protein	Nucleic Acid	Carbohydrate
Kansas City I	93.5	0.5	5.9
Kansas City II	97.7	0.9	1.3
Battey I	96.8	0.9	2.1
Battey II	94.2	2.1	3.7
Nocardia	99.1	0.05	0.8

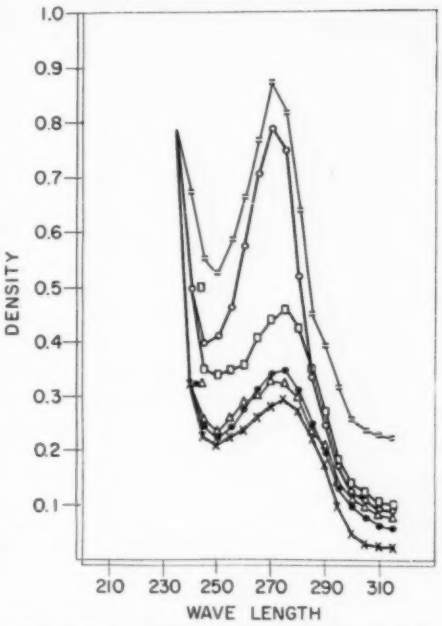


FIG. 6. Spectral absorption of PPD preparations from atypical acid-fast bacilli and that of PPD-S. ●—● Kansas City I PPD; △—△ Kansas City II PPD; □—□ Battey I PPD; ○—○ Battey II PPD; //—// Nocardia PPD; and ×—× PPD-S.

TABLE 3

ULTRAVIOLET ABSORPTION VALUES OF PPD PREPARATIONS

PPD (0.25 mg. per ml.)	Specific Absorption at Maximal Wavelength
Kansas City I	1.38
Kansas City II	1.28
Battey I	1.84
Battey II	3.17
Nocardia	3.48
PPD-S	1.16

TABLE 4

CROSS PRECIPITIN REACTIONS WITH PPD PREPARATIONS FROM ATYPICAL ACID-FAST BACILLI

Rabbit Sensitized with	Kansas City I PPD				Kansas City II PPD				Battey I PPD				Battey II PPD				Nocardia PPD				107 II (*A* Protein)			
	50*	10	5	1	50	10	5	1	50	10	5	1	50	10	5	1	50	10	5	1	50	10	5	1
Kansas City I	8	7	5	1	6	3	2	1	6	4	3	1	7	5	4	2	1	1	1	–	6	6	5	–
Kansas City II	7	6	5	2	5	3	2	–	6	3	3	–	6	4	3	1	6	3	1	–	6	6	5	–
Battey I	7	6	3	–	5	2	1	–	7	5	3	2	7	6	5	3	1	1	–	–	7	6	5	1
Battey II	6	5	4	–	4	2	1	–	6	5	4	1	7	6	5	2	1	–	–	–	6	6	4	1
Nocardia	2	1	–	–	1	–	–	–	3	2	2	1	4	3	2	1	6	5	4	2	1	–	–	–
BCG	7	5	2	–	5	2	1	–	6	5	4	2	7	6	6	3	4	3	1	1	6	6	5	2

* γ of protein.

mus nucleic acid as the standard. The PPD preparations from the two Kansas City strains and the Battey I strain showed a percentage of nucleic acid ranging from 0.5 per cent to 0.9 per cent, whereas the percentage of nucleic acid from the Battey II strain PPD was more than twice as great, while that of the Nocardia PPD was about one-tenth as great.

Ultraviolet absorption curves of the five PPD preparations were compared with those of the PPD-S (figure 6). The solutions analyzed contained 0.25 mg. of protein per ml. All of the curves were similar and showed maximal absorption at 270 to 275 mμ.

The specific absorption values (table 3) of the five PPD preparations, obtained by dividing the absorption at maximal wavelength by the protein concentration, ranged from 1.28 (Kansas City II PPD) to 3.48 (Nocardia PPD). They were all higher than for PPD-S (1.16). The high specific density given by Battey II PPD (3.17) was not unexpected since the diphenylamine reaction for desoxyribonucleic acid had shown a high content (2.1 per cent). However, the very high spectral density given by the Nocardia PPD (3.48) did not correspond to its low desoxyribonucleic acid content of 0.05 per cent obtained by chemical analysis. This variance suggested that the nucleic acid may not all be in the form of desoxyribonucleic acid but, rather, that some may be in the form of ribonucleic acid. This supposition was confirmed when an analysis was made by the orcinol reaction, and it was found that the Nocardia PPD contained sixty times as much ribonucleic acid (3.1 per cent) as desoxyribonucleic acid. This could thus account for the very high specific density value which was observed.

Precipitin tests: Precipitin tests were done to see if there were cross-reactions among the different PPD preparations and thus to demonstrate whether these fractions shared a common antigen or antigens. In table 4 are given the cross precipitin reaction between the PPD preparations and the different antisera from rabbits sensitized with each of these atypical strains as well as with the BCG strain. The concentration of protein contained in each of the antigens ranged from 1 to 50 γ, as shown in the table. The figures represent the amount of precipitate that was formed, estimated on a scale ranging from negative to 8. In addition to the PPD prepared from the atypical strains, an *A* protein which was obtained through the courtesy of Dr. F. B. Seibert and prepared by her from a human strain of tubercle bacilli was also included in this series to test the possibility of cross-reactions with it. All of the PPD preparations, as well as the *A* protein from the human strain, reacted with the sera of rabbits sensitized to all of the atypical as well as the BCG strains.

In the dilutions used, all of the PPD preparations cross-reacted with each other, thus apparently furnishing no evidence of protein specificity for each of the strains. However, it may be seen in table 5 that, when the precipitin test was carried out using much higher dilutions than were previously employed, a protein specificity became apparent. To demonstrate this, dilutions of 1:1,000,000 to 1:20,000,000 were made. It was noted that the highest titer always occurred with the PPD and its homologous antisera except for the Kansas City II PPD. This observation suggests that these strains of atypical acid-fast bacilli can be differentiated serologically.

TABLE 5

PRECIPITIN TITERS OF SERA OF RABBITS SENSITIZED WITH ATYPICAL ACID-FAST BACILLI

Rabbits Sensitized with	Kansas City I PPD						Kansas City II PPD						Battey I PPD						Battey II PPD						Nocardia PPD					
	1:1,000,000	1:2,000,000	1:5,000,000	1:10,000,000	1:15,000,000	1:20,000,000	1:1,000,000	1:2,000,000	1:5,000,000	1:10,000,000	1:15,000,000	1:20,000,000	1:1,000,000	1:2,000,000	1:5,000,000	1:10,000,000	1:15,000,000	1:20,000,000	1:1,000,000	1:2,000,000	1:5,000,000	1:10,000,000	1:15,000,000	1:20,000,000	1:1,000,000	1:2,000,000	1:5,000,000	1:10,000,000	1:15,000,000	1:20,000,000
Kansas City I	2	1					tr						1						1											
Kansas City II	tr*						tr						tr						2	1										
Battey I													1	tr					2	1										
Battey II													1	tr					2	1										
Nocardia asteroides																									1	1				

* tr = trace.

Agar-gel studies: Since cross precipitin reactions were found among the PPD preparations, they were studied by the agar-gel technique in an attempt to detect the number of antigens present in each fraction. The positions of the precipitation bands are given in table 6, using as antigens (3 mg. per ml.) the Kansas City PPD, the Battey PPD, the Nocardia PPD, and PPD-S. The numbers in the table represent the positions in millimeters from the serum-agar interface at which bands appeared and, when the band was sharp, an "s" was added. Readings were made after three, four, and five days; but it was found that some bands appeared later than others and that after ten days the maximal number of bands had formed. It may be seen that one band repeatedly appeared near the middle of the agar layer and another always appeared at the agar-serum interface. Battey I PPD gave a third band close to the antigen-agar interface with all of the antisera except with the Nocardia antiserum. Battey II PPD gave similar bands but only with Battey antisera.

Potency tests: The potencies of the PPD preparations and PPD-S given in equivalent dosages were compared in rabbits sensitized with the atypical organisms and the BCG strain and in guinea pigs which were sensitized with human tubercle bacilli. In table 7 are assembled the data of rabbits sensitized with the five atypical organisms and subsequently tested with comparable doses of the homologous PPD and PPD-S. Readings were made after twenty-four and forty-eight hours. Larger skin reactions were given with the PPD homologous with the strain used for sensitization than with PPD-S, indicating that the homologous PPD was specific. Rabbit No. 4 was an exception. The reactions to the homologous PPD and PPD-S in this rabbit were equal.

The results on rabbits sensitized with BCG and then skin-tested with each of the PPD preparations and PPD-S are given in table 8. It may be seen that in each case the larger skin reactions were elicited by PPD-S while Nocardia PPD gave the smallest reactions. Reactions were thus given by each of the PPD preparations tested, and cross-sensitization was therefore evident.

Further evidence of cross-sensitization was demonstrated on guinea pigs which had been sensitized previously with human tubercle bacilli. In table 9 are assembled the data on guinea

TABLE 6

POSITION OF PRECIPITATION BANDS WITH PPD PREPARATIONS FOUND BY THE AGAR GEL TECHNIQUE

Antisera	Antigens																					
	Kansas City I PPD				Kansas City II PPD				Battey I PPD				Battey II PPD				Nocardia PPD				PPD-S			
Mm. from serum meniscus	0	1-3	4-6	7-9	0	1-3	4-6	7-9	0	1-3	4-6	7-9	0	1-3	4-6	7-9	0	1-3	4-6	7-9	0	1-3	4-6	7-9
Kansas City I	0	3	5		0	3	6		0	5	7		0			9	0				0	1	6	
Kansas City II	0		5		0		6		0	6	8tr*		0			9	0	3tr			0		6	
Battey I	0	1	5		0		4		0	6	8		0	5		8	0				0		4.6	
Battey II	0s*		5		0s		5		0s	5	7		0s	3		9	0s				0s	3	6	
Nocardia asteroides	0				0				0				0				0		4	7	0			
BCG	0s	3	5		0s		4		0s	5	7		0s	4		8	0s				0s	2	6	

Figures represent distance in millimeters from serum meniscus at which a precipitation band occurred.

* tr = trace. s = sharp.

TABLE 7

SKIN TESTS WITH HOMOLOGOUS PPD PREPARATIONS AND PPD-S IN SENSITIZED RABBITS

Rabbit Number	Sensitized with	Dimensions of Reactions in Mm. with Homologous PPD		Dimensions of Reactions in Mm. with PPD-S	
		24 Hours	48 Hours	24 Hours	48 Hours
5	Kansas City I	22 × 22 × 2	22 × 24 × 2	15 × 14 × 1	15 × 16 × 1
6	Kansas City I	20 × 15 × 2	17 × 15 × 2	—	—
2	Kansas City II	20 × 20 × 1	20 × 20 × 1	15 × 15 × 1	15 × 15 × 1
4	Kansas City II	15 × 24 × 1	15 × 25 × 1	15 × 23 × 1	15 × 25 × 1
10	Battey I	27 × 23 × 4	35 × 37 × 4	21 × 21 × 2	22 × 23 × 2
11	Battey I	26 × 24 × 3	32 × 36 × 3	22 × 16 × 1	20 × 20 × 1
8	Battey II	46 × 41 × 2	41 × 46 × 3	22 × 22 × 1	21 × 21 × 2
9	Battey II	41 × 37 × 2	41 × 39 × 2	22 × 17 × 1	20 × 20 × 1
14	*Nocardia asteroides*	35 × 21 × 3	35 × 17 × 2	11 × 13 × 1	10 × 10 × 1
17	*Nocardia asteroides*	22 × 20 × 4	17 × 14 × 3	11 × 7 × 1	5 × 5 × 1

pigs which were simultaneously skin-tested with equivalent doses of the PPD preparations and PPD-S and were then read twenty-four hours later. The sites of the injections were randomized so that the reader did not know their identity. In each case, the largest skin reactions were given by PPD-S and the smallest by the Nocardia PPD.

DISCUSSION

That there were differences in the cultural appearance of the five discussed organisms when they were grown on both liquid and solid media has been demonstrated. In general, the two Kansas City strains gave soft, creamy growths which were cream to white in color on Long liquid medium. Moreover, this growth was characteristically different from that given by either of the two Battey strains. Similarity of cultural appearance was not observed with the two Battey strains, since Battey I strain was buff, soft, and tenacious, while Battey II strain was yellow and granular. Microscopically, the Kansas City organisms were long, slender, acid-fast rods which showed much beading, while the Battey organisms were short, coccoid-like forms. There were also differences in regard to the consistency of the dried bacillary bodies. Those of the two Kansas City strains and the Battey I strain were gummy in consistency, while the Battey II

strain was powdery and the *Nocardia asteroides* was hard and crumbly.

In addition to the protein, the PPD preparations contained small amounts of carbohydrate and nucleic acid. Although these amounts were small compared to the total amount of protein present, an analysis of the quantities of these substances would enable the detection of differences and similarities which differentiate the strains from which the PPD preparations were made. It is known that the carbohydrate present in tuberculin is in the form of two polysaccharides, Polysaccharide I and Polysaccharide II (21). Experiments in this laboratory have indicated that these polysaccharides may also be present in the filtrates from these atypical organisms. Additional studies are in progress to define more clearly the differences and similarities which have been noted in the polysaccharides from these atypical strains.

The PPD preparations also differed in their nucleic acid content. The high nucleic acid content as determined by chemical analyses for both the Battey II and the Nocardia PPD preparations explains the fact that their maximal wavelength was at 270 mμ instead of 280 mμ which is characteristic of protein. The findings are in agreement with those of Seibert (22) who showed that, when nucleic acid was added to the tuberculin protein, a definite shift occurred in wavelength of the maximal densities and a high specific density resulted. Similarly, a high specific density was found with the Battey II PPD and the Nocardia PPD (3.17 and 3.48, respectively), thus confirming spectrophotometrically that which was obtained by chemical analysis.

Each of the PPD preparations from these

TABLE 8

SKIN TESTS IN RABBITS SENSITIZED WITH BCG

Rabbit Number	PPD Tested	Dimensions of Reactions in Mm.	
		24 Hours	48 Hours
12	PPD-S	26 × 21 × 2	18 × 20 × 2
	Kansas City I	15 × 15 × 2	12 × 12 × 2
	Kansas City II	15 × 14 × 2	10 × 10 × 1
	Battey I	24 × 25 × 2	10 × 14 × 2
	Battey II	19 × 20 × 2	12 × 13 × 1
	Nocardia asteroides	10 × 7 × 2	10 × 10 × 1
13	PPD-S	37 × 26 × 2	26 × 21 × 3
	Kansas City I	16 × 16 × 2	14 × 15 × 2
	Kansas City II	21 × 16 × 2	12 × 15 × 2
	Battey I	30 × 25 × 2	20 × 20 × 2
	Battey II	20 × 15 × 2	10 × 10 × 1
	Nocardia asteroides	16 × 15 × 2	13 × 17 × 2

TABLE 9

SKIN TEST WITH PPD PREPARATIONS AND PPD-S IN SENSITIZED GUINEA PIGS

Guinea Pig Number Sensitized with Tubercle Bacilli	Dimensions of Reactions in Millimeters with 0.005 Mg. of					
	PPD-S	Kansas City I PPD	Kansas City II PPD	Battey I PPD	Battey II PPD	Nocardia PPD
793	21 × 21 × 2	15 × 16 × 2	15 × 15 × 2	10 × 10 × 1	10 × 11 × 1	5 × 5 × tr*
789	16 × 20 × 2	10 × 16 × 1	5 × 6 × 1	6 × 10 × 1	—	—
795	16 × 17 × 1	10 × 17 × 2	10 × 12 × 1	11 × 13 × 1	17 × 14 × 1	3 × 6 × tr
796	16 × 19 × 2	12 × 10 × 1	10 × 11 × 1	—	—	—
800	20 × 30 × 2	9 × 8 × 1	12 × 12 × 2	15 × 11 × 2	17 × 15 × 2	5 × 3 × tr
820	11 × 17 × 2	9 × 11 × 1	5 × 7 × 1	6 × 9 × 1	8 × 8 × 1	3 × 6 × tr
61	20 × 19 × 3	11 × 15 × 2	15 × 15 × 2	11 × 12 × 2	18 × 15 × 2	9 × 8 × 1
699	22 × 24 × 3	20 × 20 × 2	20 × 19 × 2	16 × 20 × 2	25 × 18 × 2	10 × 11 × 1
71	23 × 20 × 3	12 × 19 × 2	16 × 10 × 2	10 × 17 × 2	15 × 16 × 2	5 × 7 × 1
74	15 × 15 × 1	10 × 6 × 1	10 × 11 × 1	11 × 15 × 1	10 × 11 × 1	—
669	20 × 20 × 3	14 × 12 × 2	11 × 12 × 2	16 × 16 × 2	17 × 15 × 2	11 × 7 × 1
780	20 × 22 × 3	20 × 20 × 2	20 × 21 × 2	13 × 17 × 2	14 × 24 × 2	11 × 11 × 1
Average	19 × 20 × 3	13 × 14 × 2	13 × 13 × 2	11 × 13 × 2	13 × 13 × 2	6 × 5 × 1
Controls						
807	3 × 3 × tr	3 × 3 × tr	3 × 3 × tr	5 × 5 × tr	5 × 7 × tr	tr
812	2 × 2 × tr	3 × 3 × tr	3 × 5 × tr	6 × 6 × tr	tr	5 × 5 × tr
Average	3 × 3 × tr	4 × 3 × tr	3 × 4 × tr	6 × 6 × tr	3 × 4 × tr	3 × 3 × tr

* tr = trace.

atypical acid-fast bacilli was found to contain desoxyribonucleic acid by means of the very specific diphenylamine reaction. Petrik (23) also had shown that the extracts from six strains of atypical acid-fast bacilli contained desoxyribonucleic acid. Although all of the PPD preparations contained desoxyribonucleic acid, most of the nucleic acid in the Nocardia PPD was apparently in the form of ribonucleic acid.

Cross precipitin reactions were found between the PPD preparations and the antisera prepared against each of the atypical strains, indicating that these fractions share some antigens in common. These cross-reactions were also shown by the agar-gel technique, as indicated by the number and position of bands which appeared when the antigens were tested against the antisera. This probably accounts for the apparent lack of serologic specificity of the PPD preparations when the low dilutions of 50 to 1 γ of protein per 0.1 ml. of serum were used in the precipitin tests, since any one of the common cross-reacting components can produce a positive precipitin test. However, it was shown that it was possible by the precipitin test to differentiate the atypical acid-fast organisms from each other, except the Kansas City II strain, when higher dilutions ranging from 1:1,000,000 to 1:20,000,000 were used, since the highest titer always occurred with the PPD and its homologous antiserum. This supports the recent report of Nassau and associates (24), who used serologic means as an aid in identifying atypical Mycobacteria of the yellow bacillus type and is in accord with the work of Seibert (25) who showed that the protein made from the tuberculins of human, bovine, and timothy bacilli could be differentiated by means of the precipitin test. Thus it appears that the antigens here studied show species specificity in very high dilution.

Not only did the agar-gel method give good agreement with the precipitin test in demonstrating the antigenic relationships of the PPD preparations, but it was also an ideal method which could be used to visualize and to differentiate the antigens present in each fraction. It is possible that a band which appears in the agar layer may mask another antigen-antibody reaction which occurs in the same position, and thus the number of bands which form represent the minimal number of antigen-antibody reactions present. Therefore, since all of the PPD preparations showed two or three bands with antisera prepared against each of the atypical strains and the BCG strain, it can be concluded that these fractions consisted of a minimum of two or three different antigens.

The fact that the two PPD preparations from the Kansas City strains gave almost identical bands with their homologous antisera, while the PPD preparations from the two Battey strains showed bands in these same positions, as well as an additional component close to the antigen-agar interface, suggests that the two strains of atypical acid-fast organisms isolated in Kansas City showed some differences but also had some similar antigenic relationships to the two strains isolated in the Battey institution.

It is evident that these agar-gel studies not only demonstrated the antigenic complexity of these fractions, but also that these atypical strains are related to the human strain, since cross-reactions readily occurred with the fractions prepared from it. Similar antigenic relationships have been reported by Parlett and Youmans (26) between ninety-eight strains of Mycobacteria.

All of the PPD preparations showed a definite degree of specificity since, in the rabbits sensitized with each of the atypical organisms, the degree of skin sensitivity was much greater to the homologous PPD than to PPD-S. Similarly, rabbits sensitized with BCG gave larger skin reactions with PPD-S but were capable of producing reactions also to the heterologous PPD preparations. Additional evidence for cross-sensitization was shown in guinea pigs sensitized with human tubercle bacilli. The most potent fraction when compared with the other PPD preparations was the homologous PPD-S, but cross-sensitization was definitely demonstrated.

Summary

Purified protein derivatives (PPD) were prepared from four strains of atypical acid-fast bacilli and from one strain of *Nocardia asteroides*, using new glassware and apparatus for each strain. These organisms included two strains isolated from the Kansas City area, two strains from Battey State Hospital, Rome, Georgia, and a *Nocardia asteroides* from Duke University Medical Center.

Growth on Long liquid medium was distinctive for each strain. Differences were noted in pigment production and other colonial characteristics

when these organisms were grown on two different types of solid medium.

The Kansas City strains showed similar microscopic morphology which differed from that of the two Battey strains. In addition to the protein the PPD preparations contained small amounts of carbohydrate and nucleic acid. Ultraviolet absorption studies of the PPD preparations revealed a maximal absorption of 270 to 275 mμ. Although all of the PPD preparations contained desoxyribonucleic acid, most of the nucleic acid in the Nocardia PPD was apparently in the form of ribonucleic acid.

The PPD preparations cross-reacted with antisera prepared against each of the atypical strains and the BCG strain. Some protein specificity was shown with the PPD preparations since the highest titer usually occurred with the PPD and its homologous antisera when high dilutions were used in the precipitin tests.

Agar-gel studies showed the presence of two or three bands with most PPD preparations, and from this it may be concluded that the PPD preparations contained several antigens. The present studies demonstrated not only the antigenic complexity of these fractions but also that these atypical strains are related to some extent to the human strain, since cross-reactions readily occurred.

Additional evidence for specificity was demonstrated in laboratory animals sensitized with human tubercle bacilli and with these atypical acid-fast bacilli, since the largest skin reactions were elicited by the PPD homologous with the strain used for sensitization. However, there was a cross skin sensitization to all of the PPD preparations.

Sumario

El Derivado Proteínico Purificado (DPP) y Otros Antígenes Preparados de Atípicos Bacilos Acidorresistentes y Nocardia Asteroides

De cuatro cepas de atípicos bacilos ácidorresistentes y de una cepa de *Nocardia asteroides* se prepararon derivados proteínicos purificados (DPP), usando nuevos aparatos y cristalería para cada cepa. Estos gérmenes comprendían dos cepas aisladas de la zona de Kansas City, dos procedentes del Hospital-Hogar Battey del Estado de Georgia y una *Nocardia asteroides* del Centro Médico de la Universidad de Duke.

La proliferación en el medio líquido de Long fué típica para cada cepa. Al cultivarse estos microbios en dos distintas clases de medios sólidos, notáronse diferencias en la producción de pigmento y otras características de las colonias.

Las cepas de Kansas City revelaron una morfología microscópica semejante que discrepaba de la de las dos cepas de Battey. Además de la proteína, los DPPs contenían pequeñas cantidades de hidratos de carbono y de ácido nucleico. Los estudios de la absorción de rayos ultravioletas por los DPPs revelaron una absorción máxima de 270 a 275 μ. Aunque todos los DPPs contenían ácido desoxirribonucleico, la mayor parte del ácido nucleico en el DPP de la Nocardia tomaba aparentemente la forma de ácido ribonucleico.

Los DPPs mostraron una reacción cruzada con los antisueros preparados contra cada una de las cepas atípicas y la cepa BCG. Existía alguna especificidad para proteínas en los DPPs, dado que solió observarse el título más alto en el DPP y sus antisueros homólogos cuando se usaban diluciones altas en la precipitinorreacciones.

Los estudios en gel-agar revelaron la presencia de dos o tres bandas con la mayoría de los DPPs, de lo cual cabe deducir que éstos contenían varios antígenos. Los estudios actuales demostraron no sólo la complejidad antigénica de estas fracciones, sino también que estas cepas atípicas guardan cierta relación con la cepa humana, pues se produjeron fácilmente reacciones cruzadas.

So observaron nuevas pruebas de especificidad en los animales de laboratorio sensibilizados con bacilos tuberculosos humanos y con estos bacilos ácidorresistentes atípicos, visto que las cutirreacciones mayores fueron provocadas por el DPP homólogo usado para la sensibilización. No obstante, hubo una cutisensibilización cruzada a todos los DPPs.

Resume

Dérivés protéiques purifiés (PPD) et autres antigènes préparés a partir de bacilles acido-résistants et de Nocardia astéroides atypiques

On a préparé des dérivés protéiques purifiés à partir de quatre souches de bacilles acido-résistants et d'une souche de *Nocardia asteroides* en utilisant pour la préparation de chaque souche de la verrerie et du matériel neufs. Ces micro-organismes comprenaient deux souches isolées dans la région de Kansas City, deux souches dans le Battey State Hospital de Rome, Georgia (U.S.A.) et une de *Nocardia asteroides* de Duke University Medical Center.

En milieu liquide de Long, chaque souche présentait une croissance distincte. Des différences ont été enregistrées quant à la production de pigment et diverses caractéristiques de colonisation

quand ces germes étaient cultivés sur deux milieux solides différents.

Les souches de Kansas City présentaient une morphologie microscopique similaire différant de celle des deux souches de Battey. Outre la protéine, le PPD renfermait de petites quantités de glucide et d'acide nucléique. Les études sur l'absorption des PPD dans l'ultra-violet ont révélé une absorption maximum de 270 à 275 μ. Tous les dérivés protéiques purifiés contenaient de l'acide desoxyribonucléique, mais la plus grande partie de l'acide nucléique de PPD de *Nocardia* existait sous la forme d'acide ribonucléique.

Les PPD présentaient une réaction croisée avec les antisérums préparés contre chaque souche atypique et la souche BCG. Une certaine spécificité protéique s'observait avec les PPD, car les titres les plus élevés apparaissaient avec le PPD et ses antisérums homologues quand de hautes dilutions étaient utilisées dans les précipitine tests.

Les études en milieu gel agar révélaient la présence de deux ou trois bandes avec la plupart des PPD; cela a permis de conclure que les PPD renfermaient plusieurs antigènes. Ces études démontrent non seulement la complexité antigénique de ces fractions, mais aussi que ces souches atypiques sont à un certain point apparentées à la souche humaine, étant donné que les réactions croisées se produisaient facilement.

D'autres preuves de spécificité ont été démontrées chez l'animal expérimental sensibilisé avec des bacilles tuberculeux humains et avec ces bacilles acido-résistants, car les réactions cutanées les plus importantes ont été provoquées par les PPD homologues des souches utilisées pour la sensibilisation. Toutefois, la sensibilisation croisée cutanée se manifestait avec tous les PPD.

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SOME BIOLOGIC PROPERTIES OF MYCOBACTERIA FOLLOWING THE EXTRACTION OF LIPIDS[1,2,3]

The Power to Initiate Acquired Resistance

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INTRODUCTION

The writers have previously reported on some biologic properties of Mycobacteria following the extraction of lipids (1) and have shown that, using carefully calculated comparable doses, the progressive extraction of the lipids of BCG yielded bacillary residues showing a reduced power to irritate tissues, to prepare for and to evoke Bail's phenomenon,[4] and to sensitize to tuberculin. Consequently, such residues might be administered in large doses to study any acquired resistance they may initiate in experimental animals. Evidence brought forth by Weiss and Dubos (2), suggesting that "toxicity and protective activity were independent one from the other and were the manifestation of different bacillary constituents," gave countenance to the present investigation to discover if the immunizing properties of such bacillary residues remained impaired even though the power to irritate the tissues, to prepare for and evoke Bail's phenomenon, and to sensitize to tuberculin had been appreciably reduced.

Review of the Literature

Discrepancies were found in the literature with respect to the biologic properties of bacillary residues. In 1936, Macheboeuf and Dieryck (3) showed that rabbits could be immunized against an experimental tuberculous infection by a single injection of tubercle bacilli extracted with acetone and ether. Ten years later, Raffel (4), working with guinea pigs, reported that acquired resistance in tuberculosis could not be ascribed to any specific chemical portion of tubercl bacilli or to defatted bacilli even when injected in massive doses. More recently, Weiss and Dubos (2) have shown that mice could indeed be vaccinated with BCG microorganisms killed with phenol and extracted with chloroform and monochlorobenzene, or with phenol-killed and methanol-extracted avirulent bacilli H37Ra. Smith and Kubica (5) showed almost simultaneously that an antituberculous resistance had taken place in guinea pigs vaccinated with small amounts of extracted bacilli suspended in adjuvant. The latter writers have even stated that guinea pigs vaccinated with whole dead bacilli were not protected to the same extent, results that would imply that the vaccine was improved by removing neutral solvent-soluble substances.

Even though attention has been drawn to the biologic properties of extracted bacilli by Raffel, Weiss and Dubos, and Smith and Kubica, no systematic study of the biologic properties of progressively more fully extracted bacillary residues has been made, the main objective of these writers being the properties of the extracts rather than those of the residues. The aim of the present project was to discover precisely to what extent guinea pigs could be immunized with varying doses of bacillary residues with progressively decreasing lipid content. Even though adjuvants had generally been used by the above writers to suspend the extracted bacilli, it has been judged preferable not to use them in this first series of experiments on acquired resistance, but to delay their use to forthcoming experiments with the preparations that would turn out to be the most promising in the present experiment. The recent publication of Hoyt and associates (6), stating: "The possibility of killing tubercle bacilli by the first step in a series of mild extractions, and testing the antigenicity of such killed organisms (both with and without adjuvants) following each extractive procedure, particularly interests the present investigators," urges the present writers

[1] From the Institute of Microbiology and Hygiene and the Center of Statistics of the University of Montreal, Montreal, Canada.

[2] Supported, in part, by a grant from the Ministry of Health of the Province of Quebec (Federal-Provincial Health Research Grants).

[3] Fourth report on "Studies on Mycobacteria." For the third report, see (1).

[4] In guinea pigs highly sensitized with tubercle bacilli, Bail has described a reaction to the intraperitoneal introduction of bacillary bodies characterized by an immediate leukocytic response and death within a few hours (Bail, O.: Wien. klin. Wchnschr., 1904, *17*, 846). The present investigators applied a somewhat similar but more quantitative technique, and the survival rate was the only criterion used.

to publish their results. The present paper reports on the immunizing properties for guinea pigs of progressively more fully extracted bacillary residues suspended in saline.

Materials and Methods

Culture and harvesting of the bacteria: Mycobacterium tuberculosis var. *bovis BCG* and the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* were used throughout this project. Both the attenuated and virulent strains were grown on Sauton's medium at 38°C. and harvested after seven and twenty-eight days, respectively, of incubation as previously described (1).

Extraction of the lipids: Two series of bacillary residues were studied. The first one arose from the extraction of BCG according to Macheboeuf's method (7) and consisted of five bacillary residues, progressively more fully extracted and designated BCG residue I,—V (1). The second series resulted from the extraction of the H37Rv strain by two different techniques. Half of the H37Rv harvest was extracted by acetone and ether, as described by Macheboeuf and Dieryck (3), thus yielding an incompletely extracted bacillary residue designated "chemical vaccine." The other half of the H37Rv crop was exhaustively extracted with a mixture of ethanol-ether (1:1) followed by chloroform; the resulting bacillary residue, deprived of free lipids, was called "H37Rv residue."

Preparation of the suspensions of bacillary residues: All of the residues were lyophilized to remove any trace of solvent, and the required amount for each experiment was homogenized with a few milliliters of sterile saline in a TemBroeck apparatus. The homogenate was diluted with saline to the desired concentration. Suspensions of bacillary residues retained their homogeneity for so short a time that they had to be prepared immediately before injection. Only a single dose was taken into the syringe at one time and between each injection the suspension was homogenized by forcing it in and out of the syringe.

Preparation and control of the killed BCG vaccine: BCG at a 90-mg. per ml. concentration was put in ampoules and heated at 60°C. for one hour in a water-bath. A sterility test on Löwenstein medium revealed no colony either for the killed BCG vaccine or for the various bacillary residues.

Animals: All experiments were carried out with random-bred tuberculin-negative guinea pigs weighing approximately 400 gm. They were divided at random into groups of 25 or 30 pigs each, half male and half female, and were housed in groups of 5 in metal cages. The diet of the animals before and during challenge consisted of pellets, vegetables, and tap water ad libitum.

Experimental procedure: After a fourteen-day period of observation, the experiment was begun. The protocol for each *in vivo* experiment was as follows: Guinea pigs were vaccinated with various doses of bacillary residues by the routes of injection described in the section "Results." A group of BCG-vaccinated animals and a nontreated control group were included in each experiment. Three months after the last vaccination, all animals were shaved and tuberculin tested in the right lower quadrant of the abdomen with 10 mg. of Old Tuberculin (OT) in 0.1 ml. of saline by the intracutaneous route. The results were read at forty-eight hours, a positive reaction being at least 25 mm.^{2} minimum surface of induration as determined by multiplying the transverse diameters. Following the tuberculin test, all groups were challenged subcutaneously in the left inguinal region with 0.00001 mg. moist weight of tubercle bacilli, Ravenel strain, from an eight-day-old culture in Dubos liquid medium.

All animals were regularly observed while the chronic disease developed and were examined at autopsy as they died, the presence of macroscopic tuberculous lesions on the lungs, livers, and spleens being registered. On or about the seventieth day after infection, control animals started to die with macroscopic lesions on the above cited organs. The experiment was extended till 50 per cent of the control animals had died with tuberculous lesions. For the experiments reported here, this 50 per cent survival time was between 124 and 160 days. All animals remaining in the experiment were then given numbers at random, and the key for the assigned numbers was sealed and not examined until post-mortem examination of all groups had been completed. This was done to reduce any unintentional bias which might be shown in evaluating the extent of gross disease at autopsy and to discourage any inadvertent attempt to reduce the variation from one group to the next. All of the guinea pigs were then sacrificed (chloroform), and the extent of gross tuberculosis on the lungs, spleen, and liver was estimated for each organ according to the following code values:

Code value 0: no nodules on the surface of the organ

Code value 1: 1, 2, or 3 small nodules on the surface of the organ

Code value 2: less than 50 per cent of the surface of the organ invaded by nodules and confluent lesions

Code value 3: more than 50 per cent of the surface of the organ invaded by nodules and confluent lesions

Code value 4: the surface of the organ completely covered by nodules and confluent lesions.

It may be noted that the code values 0, 1, and 4 correspond to well-defined stages of invasion of the organs and are ascribed easily. The code values 2 and 3 which, in some rare cases, correspond to neighboring stages of invasion could have been ascribed to individual organs with some risk of inversion. At all events, each organ, tagged with the number of the corresponding guinea pig, was kept overnight in 3 per cent formaldehyde. It was then placed in a dish with those of the same

code value, compared, and definitely coded. In fact, very few modifications came from this second and comparative examination. The use of the key permitted identification of the coded organs with the corresponding animal and distribution of the animals in the original experimental group. It follows that each animal with its three coded organs had an index in a triplet form, for example (2, 1, 1,) or (3, 0, 0).

Statistical method for the presentation of the results: A first tentative comparison between experimental groups could be made by averaging all of the code values in each group. However, this method could be criticized because it does not account sufficiently for the degree of severity of the tuberculous involvement in sick animals. In the former instance, an animal with index (3, 0, 0) would be classified in the same category as one with index (1, 1, 1). It was not clear whether both indices expressed equal pathologic conditions. It was then decided to arrange the indices of each experimental group of animals in the gradual scale forming the following five categories:

Category I: (0, 0, 0), (1, 0, 0), (1, 1, 0), (1, 1, 1)
Category II: (2, 0, 0), (2, 1, 0), (2, 1, 1)
Category III: (3, 0, 0), (3, 1, 0), (3, 1, 1), (2, 2, 0), (2, 2, 1)
Category IV: (3, 2, 0), (3, 2, 1), (3, 3, 0), (3, 3, 1), (2, 2, 2), (4, 2, 0)
Category V: (3, 2, 2), (3, 3, 2), et cetera.

It may thus be seen that:

Category I includes cases without any gross tuberculous lesions as well as those with a very small number of nodules.

Category II includes cases in which a single organ is rather seriously invaded by tuberculous lesions.

Category III is made up of cases in which two organs are rather seriously invaded or in which a single organ is heavily covered with lesions.

Category IV corresponds to cases in which two organs are heavily invaded or in which three organs are seriously invaded by lesions.

Category V is made up of all of the other more seriously attacked cases.

This grouping is arbitrary, as any such grouping would be. However, it may more logically represent the natural progression of the gross lesions. At the beginning, statistical analysis will take this grouping into account and, later on, in accordance with the requirements set by the special studies, other partitions that will combine some categories will also be taken into consideration.

The most powerful non-parametric method used in this type of study is known as the chi-square test. The validity of this test is based on the following restriction: in each category, for a given "treatment" (experimental group), the average number of observations must be at least four. This restriction must also be applied to each "treatment" (experimental group) in a given category.

Results

Experiment No. 1

One hundred and twenty-five guinea pigs were divided into five experimental groups of 25 animals each. One untreated group (*Group 1*) served as controls and the other four received six scarifications on the depilated back through drops of the following suspensions:

Group 2: 60 mg. per ml. (moist weight) of standard living fresh BCG suspension, which is the standard concentration used by the Institute of Microbiology and Hygiene of the University of Montreal for human transcutaneous vaccination.

Group 3: 13 mg. per ml. suspension of BCG residue I (BCG extracted with cold acetone). This concentration is the proportion of residue I in a 60 mg. per ml. suspension of standard BCG.

Group 4: a suspension of 12.35 mg. of BCG residue I (that is the proportion of residue I in a 60 mg. per ml. suspension of standard BCG, less 5 per cent) plus 3 mg. of fresh standard BCG per ml. This total suspension contains 5 per cent standard BCG and was included in the experiment to discover the effect of small amounts of standard BCG injected simultaneously with BCG residue I.

Group 5: a suspension of 3 mg. per ml. of fresh standard BCG or 5 per cent of the concentration used in *Group 2*. This group served as a specific control to *Group 4*.

The pigs were treated thereafter as described in the experimental procedure. The scheme of this first experiment is presented in table 1, together with some of the results obtained with the above proportional doses of BCG residue I. It may be seen that 22 per cent of the animals injected with these doses of BCG residue I gave reactions to 10 mg. of OT. The pigs treated with BCG residue I plus 5 per cent of the standard BCG reacted in the same way as those immunized with BCG alone, whether in a 3 or 60 mg. per ml. suspension.

One hundred and twenty-four days after infection, 72 per cent of the animals injected with the BCG residue I were still alive as compared with 50 per cent of the control animals. The highest survival rates were obtained in the groups of animals immunized with BCG, whether alone or mixed with BCG residue I.[5]

The grouping of the lesion indices of the sacrificed animals in the various categories of indices is shown in table 2. The following conclusions

[5] The significance of the relative survival rates in all three experiments here reported will be explained in the "Discussion."

TABLE 1

EXPERIMENT NO. 1

IMMUNIZATION WITH A RELATIVELY LOW CONCENTRATION OF BCG RESIDUE I

Results of Survival and Hypersensitivity Tests

Groups of Animals*	Scarification with Suspensions of Mg. per Ml.	Per Cent Positive Reactions to 10 Mg. OT†	Number of Guinea Pigs					Per Cent of Survival at 124 Days
			Died from Intercurrent Infections		Kept in Experiment	Died with Tuberculous Lesions	Sacrificed 124 Days After Infection	
			Before Infection	After Infection				
1-Controls	Nothing	0	4	3	18	9	9	50.0
2-Standard BCG	60	100	4	2	19	2	17	89.5
3-BCG residue I	13	22	1	2	22	6	16	72.7
4-BCG residue I plus 5% standard BCG	12.35 residue I +3 BCG	100	1	2	22	3	19	86.4
5-5% standard BCG	3	100	3	2	20	0	20	100.0

* 25 guinea pigs per group.

† Positive reaction: 25 mm.² minimum surface of induration. Reaction performed 3 months after immunization and read at 48 hours.

TABLE 2

EXPERIMENT NO. 1

IMMUNIZATION WITH A RELATIVELY LOW CONCENTRATION OF BCG RESIDUE I

Grouping of the Lesion Indices of the Sacrificed Animals in the Various Categories

Groups of Animals / Categories	Number and Per Cent of Guinea Pigs in								Total
	I		II	III	IV	V	Total in II + III + IV + V		
	Number	Per Cent					Number	Per Cent	
1-Controls	4	44.4	0	1	3	1	5	55.6	9
2-Standard BCG	12	70.6	3	0	1	1	5	29.4	17
3-BCG residue I	3	18.7	2	4	3	4	13	81.3	16
4-BCG residue I plus 5% standard BCG	13	68.4	3	2	1	0	6	31.6	19
5-5% standard BCG	16	80.0	4	0	0	0	4	20.0	20

may be drawn from the statistical analyses made with the chi-square test: The controls and the animals immunized with BCG residue I gave essentially the same results regardless of the partition of categories studied; guinea pigs immunized with the standard BCG, the BCG residue I plus 5 per cent of the standard BCG, and the 5 per cent standard BCG gave comparative results; a significant difference (at an average level of 1 per cent) was found to exist between the control group combined with the group immunized with BCG residue I, on the one hand, and the three remaining groups (all including live BCG), on the other. This difference existed for all the partitions of categories for which the test was valid.

Experiment No. 2

The first experiment showed that guinea pigs could not be protected against an experimental tuberculous infection when scarified with comparatively small doses of BCG residue I. A second experiment was thus undertaken to discover if massive doses of bacillary residues obtained from BCG organisms by using Macheboeuff's method would initiate any acquired resistance in the pigs.

Five groups of 30 animals each were used. One group (*Group 1*) served as a control and another one was scarified with standard BCG (*Group 5*). The other three groups of pigs were first injected intraperitoneally; during the next week they received a series of six scarifications, followed five

days later by a second identical series of scarifications. The following suspensions were used:

Group 2: Heat-killed BCG intraperitoneally: 1 ml. of a 90 mg. per ml. suspension.
Scarification: with a 180 mg. per ml. suspension.

Group 3: BCG residues I + II (equal parts) intraperitoneally: 1 ml. of a 20 mg. per ml. suspension.
Scarification: with a 40 mg. per ml. suspension.

Group 4: BCG residues III + IV + V (equal parts) intraperitoneally: 1 ml. of a 15 mg. per ml. suspension.
Scarification: with a 30 mg. per ml. suspension.

Because of the small amounts of bacillary residues available, these had to be combined into two series based on the results of the electron-microscopic examinations and on the decrease of the power to irritate tissues observed after methanol extraction as previously reported (1). The pigs were thereafter treated as described in the experimental procedure. The scheme of the experiment is presented in table 3, together with some of the results obtained. It may be seen that 85 per cent of the pigs injected with heat-killed BCG reacted to OT. Forty-seven per cent of those injected with BCG residues I + II and only 29 per cent of those treated with BCG residues III + IV + V gave reactions.

One hundred and twenty-four days after infection, the animals scarified with standard BCG had the highest survival rate, whereas all of the other treated pigs showed only a slight increase of survival rate over that of the controls. The grouping of the lesion indices of the sacrificed guinea pigs in the various categories of indices is summarized in table 4.

All of the statistical analysis from which comparisons between the groups could be established did not yield any truly significant differences when category I was used against categories II + III + IV + V. No other combination of the above categories yielded valid results.

However, if the following partition of categories is made for each group, i.e., animals with index (0, 0, 0), on the one hand, and all of the other pigs on the other, it may be concluded that: *Group 5* (standard BCG) was significantly different from *Group 1* (control), at a 10 per cent level; *Groups 1, 3,* and *4* seemed statistically equivalent (control, BCG residues) although *Groups 3* and *4* (BCG residues) at first sight seemed more resistant than the control group; *Group 2* (heat-killed BCG) appeared to show better results than *Groups 1, 3,* and *4* (average significant level of 15 per cent).

In summary, the groups inoculated with the BCG residues in massive doses and by intraperitoneal and scarification routes showed only an insignificant resistance to the experimental infection, whereas an appreciable level of resistance was found in those immunized with living or killed BCG.

TABLE 3

EXPERIMENT NO. 2

IMMUNIZATION WITH MASSIVE DOSES OF BACILLARY RESIDUES OBTAINED FROM THE BCG STRAIN

Results of Survival and Hypersensitivity Tests

Groups of Animals*	Injection with Suspension of Mg. per Ml.	Per Cent Positive Reactions to 10 mg. OT†	Number of Guinea Pigs					Per Cent of Survival at 124 Days
			Died from Intercurrent Infections		Kept in Experiment	Died with Tuberculous Lesions	Sacrificed 124 Days After Infection	
			Before Infection	After Infection				
1-Controls	Nothing	0	6	0	24	12	12	50.0
2-Heat-killed BCG	90 mg. I.P.‡ +2 Sc. 180	85	7	4	19	8	11	58.0
3-BCG residues I+II	20 mg. I.P. +2 Sc. 40	47	0	3	27	9	18	66.6
4-BCG residues III+IV+V	15 mg. I.P. +2 Sc. 30	29	2	1	27	11	16	59.2
5-Standard BCG	Sc. 60	96	5	4	21	2	19	90.4

* 30 guinea pigs per group.

† Positive reaction: 25 mm.² minimum surface of induration. Reaction performed 3 months after immunization and read at 48 hours.

‡ I.P.: intraperitoneally; Sc.: a series of 6 scarifications with a mg. per ml. suspension.

TABLE 4

EXPERIMENT No. 2

IMMUNIZATION WITH MASSIVE DOSES OF BACILLARY RESIDUES OBTAINED FROM THE BCG STRAIN

Grouping of the Lesion Indices of the Sacrificed Animals in the Various Categories of Indices

Groups of Animals / Categories	Number and Per Cent of Guinea Pigs in										
	I				II	III	IV	V	Total in II + III + IV + V		Total
	Number in			Per Cent							
	(0, 0, 0)	(1, 0, 0) (1, 1, 0) (1, 1, 1)	Total						Number	Per Cent	
1-Controls	2	4	6	50.0	1	2	2	1	6	50.0	12
2-Heat-killed BCG	4	4	8	72.7	1	0	1	1	3	27.3	11
3-BCG residues I + II	2	9	11	61.1	3	3	1	0	7	38.9	18
4-BCG residues III + IV + V	3	7	10	62.5	1	1	3	1	6	37.5	16
5-Standard BCG	9	6	15	79.0	1	1	0	2	4	21.0	19

Experiment No. 3

A third experiment was set up to verify whether bacillary residues obtained from virulent microorganisms with a different extraction procedure, and injected by the intraperitoneal and the subcutaneous routes, would initiate any acquired resistance.

The residues used were the "chemical vaccine" and the "H37Rv residue" prepared from the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* as described in "Materials and Methods." Twenty-five milligrams of each of these residues were injected in five injections of 5 mg. each, the first injection by the intraperitoneal route and the others by the subcutaneous route at four-day-intervals.

Two preparations obtained from the BCG strain for another project were included in this experiment to verify their ability to induce acquired resistance. These were a sterile borate (pH 8.4) extract of mechanically disrupted BCG cells and a sterile filtrate of a fifty-day-old culture of BCG on Sauton's medium. Both of these preparations were included in this experiment for reasons of economy, and the results were analyzed simultaneously with those obtained with the bacillary residues. Groups of 30 guinea pigs were used in this experiment, the scheme of which is presented in table 5, together with some of the results. It may be seen that the highest level of hypersensitivity thus far reached with bacillary residues was registered for the animals treated with the "chemical vaccine" (82.7 per cent) and with the "H37Rv residue" (78.5 per cent). The BCG filtrate and the borate extract induced practically no allergy in the pigs.

The survival rates of both the former groups were 68 and 76 per cent, respectively, as compared with 50 per cent for the controls, 73.9 per cent and 50 per cent for the extract and the filtrate, and 80 per cent for the BCG-treated pigs.

The grouping of the lesion indices of the sacrificed animals in the various categories of indices is shown in table 6.

Because of the small number of animals in each of the categories, the statistical analysis had to be made with different groupings of those categories. The first, which is the most natural, is category I (animals without any gross tuberculous lesions as well as those with two or three very small nodules at the most), against the last four categories (animals with organs severely invaded by tuberculous lesions). The following conclusions may thus be drawn from this first analysis: No significant protection was induced by the borate extract or by the BCG filtrate, two groups being thus found equivalent. In fact, tuberculosis seemed to have been enhanced by these two preparations (although they induced practically no allergy) since the proportion of animals *without* lesions in these groups (5.9 and 7.7 per cent) is much less than that observed in the controls (23 per cent); no statistically detectable difference was found to exist between the "chemical vaccine" and the "H37Rv residue," two preparations which induced in the guinea pigs a certain protection, the level of which could not be validly distinguished with this regrouping from that given by BCG.

TABLE 5

EXPERIMENT NO. 3

IMMUNIZATION WITH MASSIVE REPEATED DOSES OF BACILLARY RESIDUES PREPARED FROM THE H37Rv STRAIN

Results of Survival and Hypersensitivity Tests

Groups of Animals*	Injection with Suspensions of Mg. per Ml.	Per Cent Positive Reactions to 10 Mg. OT†	Number of Guinea Pigs					Per Cent of Survival at 160 Days
			Died from Intercurrent Infections		Kept in Experiment	Died with Tuberculous Lesions	Sacrificed 160 Days After Infection	
			Before Infection	After Infection				
1-Controls	Nothing	0	4	0	26	13	13	50.0
2-Standard BCG	Sc. 60‡	100.0	10	5	15	3	12	80.0
3-Chemical vaccine	5 mg. I.P.§ + 4 × 5 mg. S. C.	82.7	2	3	25	8	17	68.0
4-H37Rv residue	5 mg. I.P. + 4 × 5 mg. S. C.	78.5	2	3	25	6	19	76.0
5-Borate extract	5 mg. I.P. + 4 × 3.5 mg. S. C.	3.8	5	2	23	6	17	73.9
6-BCG filtrate	5 mg. I. P. + 4 × 3.5 mg. S. C.	0	3	1	26	13	13	50.0

* Groups of 30 guinea pigs.

† Positive reaction: 25 mm.² minimum surface of induration. Reaction performed 3 months after the last injection and read at 48 hours.

‡ Sc.: 6 scarifications with a suspension of mg. per ml.

§ I.P.: intraperitoneally; S.C.: subcutaneously.

TABLE 6

EXPERIMENT NO. 3

IMMUNIZATION WITH MASSIVE REPEATED DOSES OF BACILLARY RESIDUES PREPARED FROM THE H37Rv STRAIN

Grouping of the Lesion Indices of the Sacrificed Animals in the Various Categories of Indices

Groups of Animals / Categories	Number and Per Cent of Guinea Pigs in								Total
	I		II	III	IV	V	Total in II + III + IV + V		
	Number	Per Cent					Number	Per Cent	
1-Controls	3	23.0	2	3	3	2	10	77.0	13
2-Standard BCG	7	58.3	3	2	0	0	5	41.7	12
3-Chemical vaccine	6	35.3	1	7	2	1	11	64.7	17
4-H37Rv residue	10	52.6	1	3	5	0	9	47.4	19
5-Borate extract	1	5.9	5	5	2	4	16	94.1	17
6-BCG filtrate	1	7.7	6	2	2	2	12	92.3	13

In order to study further the level of protection induced by the "chemical vaccine" and by the "H37Rv residue," a second grouping of categories was made. Two new groups were thus studied: the first one included animals without any or with two or three very small nodules at the most, together with those who had also a single organ seriously invaded by tuberculous lesions (animals of categories I and II); the second one included guinea pigs of categories III + IV + V. Statistical analysis performed with this second grouping gave the following

results: the "chemical vaccine" and the "H37Rv residue" groups of pigs were identical; the protection induced by these two preparations was significantly weaker (4 per cent level) than that established by BCG.

Discussion

No adjuvant was used in these experiments in order to detect, without any enhancing effect, any significant specific increase in antituberculous resistance that might have taken place. In order to detect the small differences in antituberculous resistance that might exist between the experimental groups, small infective doses of virulent tubercle bacilli were used, as recommended by several investigators (8–11). Therefore, it seemed inadvisable to attempt to evaluate the immunity according to the survival time exclusively because of the relatively long duration of this type of experiment and of the occurrence of too many intercurrent diseases. Moreover, some guinea pigs of the various experimental groups, the controls as well as the others, would undoubtedly (as happened in similar experiments in the past) have survived such a small infection far beyond the time when the average animal would have died. Groups are then disrupted. It has also been frequently observed, in the past as well as in the above experiments, that many of the animals which die naturally do not show an extent of gross tuberculous involvement sufficient to explain the death. These animals cannot be classified as dead with generalized tuberculosis, although few causes other than mechanical complications (such as hemorrhagia in serous membranes) due to tuberculous disease could be ascribed to those deaths. Survival rates are sometimes different in groups which otherwise show no difference in resistance at all.

In *Experiment No. 3*, the BCG filtrate group had the same survival rate as that of the control group, whereas the BCG extract group had a survival similar to that of the standard BCG group. Both the filtrate and the extract groups showed no protection when the lesions of the sacrificed pigs were compared. It is possible that larger groups of animals would have given more consistent results in this respect. It is therefore obvious that a challenge-survival type of experiment, using a limited number of animals and small virulent doses of tubercle bacilli in guinea pigs, may present serious inconvenience.

Hence, a challenge-sacrifice type of experiment was chosen and, as said above, it was deemed that more precise information might be gained by using a small infective dose which, in the experience of the present writers, caused death of 50 per cent of the controls within approximately 150 days. Gross tuberculous lesions of the lungs, spleen, and liver were evaluated on a scale ranging from code value 0 to code value 4; only the code values of the sacrificed pigs (when 50 per cent of the controls had died with tuberculous lesions) were analyzed, so that all animals compared would have survived the chronic disease for the same length of time. The present method of evaluating quantitatively the gross involvement of each organ seems easy, since it is limited to five figures: no visible lesions at all; only one, two, or three small nodules on the surface of the organ; lesions covering less than 50 per cent of the surface; or more than 50 per cent but not 100 per cent; and, finally, 100 per cent. The difficulty arises when the average extent of the disease for each animal and for a group of animals has to be synthetized and determined from these observations. Numerical values corresponding to empirical indices of pathogenicity have been ascribed by certain writers to one or the other organ of the infected guinea pigs (5, 12). In order to be as objective as possible, as said above, each sacrificed pig was given an index in triplet form, based on the number of gross lesions observed at the surface of the spleen, the lungs, and the liver; and all of these indices were thereafter grouped in categories in order to take into account the gravity of invasion of an individual with regard to another one. This procedure permits a more logical and positive approach to statistical analysis, especially when the chi-square test is used. It permits a wide range of possible statistical analyses, since the partition or the combination of categories may yield not only one set, but two or three sets of analyses.

All of the bacillary residues studied in this project were acid fast and less toxic in sensitized animals than the corresponding heat-killed bacilli. Varying levels of hypersensitivity were found in all of the groups of pigs treated with bacillary residue; from 22 per cent of positive reactions in the group injected with relatively small amounts of BCG residue I, hypersensitivity reached 47 per cent and 80 per cent in animals injected with massive repeated doses of residues

obtained either from BCG or from the H37Rv virulent strain. The residues never yielded any colony on Löwenstein medium. Resistance was always higher in groups showing a fair degree of allergy.

It has been shown in *Experiment No. 1* that no acquired resistance was induced in guinea pigs scarified with a single series of scarifications made with a relatively low concentration of BCG residue I (acetone extraction according to Macheboeuf's method) and that the addition of a small amount (5 per cent) of standard BCG to this residue resulted only in the protection afforded by the added BCG alone. It therefore seems that a satisfactory threshold of immunity can be obtained with a certain minimal amount of fresh BCG. Thereafter, the introduction of more standard BCG does not, according to the experience of the present writers, proportionally increase the degree of allergy (13) or the degree of immunity (14).

Experiment No. 2 presented deceiving results. No acquired resistance could be statistically proved to exist in animals injected with massive doses of bacillary residues obtained with Macheboeuf's method from the attenuated BCG strain, although some protection was obtained with massive doses of killed standard BCG. However, it was believed that those results could gain some interest from the discussion. There is no doubt that the relatively small number of animals that were still living in each group at the time of the sacrifice, as well as the too weak virulence of the challenge dose, contributed to minimizing differences between the experimental groups by reducing the number of animals in categories corresponding to extensive lesions. This shows the inconvenience of the use of small challenge doses; if the virulence of the strain unexpectedly decreases, tuberculous lesions are then less differentiated between the groups. It is not always possible to administer the optimal challenge dose. But there is some speculation whether BCG, as extracted by Macheboeuf's method, would not yield only a weak or non-immunizing antigen. With this method, bacillary bodies are kept at least thirty days in contact with each of the various solvents. In *Experiment No. 3*, the methods used were less drastic from this point of view and these residues have kept a higher power of sensitizing to tuberculin.

The statistical analysis of the results obtained in *Experiment No. 3* with massive repeated injections of "chemical vaccine" and of "H37Rv residue" has demonstrated that both of these preparations administered by one intraperitoneal dose followed by two successive subcutaneous doses initiated a significant level of protection in the guinea pigs, although this protection was significantly weaker (4 per cent level) than that induced by the standard BCG. It is possible that a challenge made earlier than three months after vaccination would have revealed a higher level of protection for the above residues. These latter have been injected without adjuvant and it is likely that they have been eliminated relatively quickly. The state of immunity may then have been quite transient. It is also to be noted that both the above residues are equally active, although the "chemical vaccine" was less completely extracted than the "H37Rv residue." Successive series of subcutaneous injections have been combined with one single intraperitoneal injection because repeated injections by this latter route might have produced undesirable sensitivity reactions and this does not immunize the guinea pig as well as the scarification or subcutaneous methods.

A more elaborate statistical analysis of the results obtained either with the relatively small dose of BCG residue I (acetone extraction-concentration corresponding to a 60 mg. per ml. concentration of standard BCG) in *Experiment No. 1*, or with massive doses of a combination of BCG residue I and II (acetone and ether extraction), tended to show that these preparations enhanced the tuberculous disease of the guinea pig. It may thus be postulated that those animals, though only 22 and 47 per cent of them reacted to tuberculin, were sensitized rather than immunized, probably by toxic lipids not yet extracted, while immunizing antigens were either too weak in quantity or destroyed. However, massive doses of a combination of BCG residues III + IV + V in *Experiment No. 2*, having sensitized 29 per cent of the animals to tuberculin, did not produce the enhancing effect mentioned above.

Summary

Saline suspensions of several bacillary residues of varying lipid contents were injected in guinea pigs to study their ability to stimulate active resistance. Gross tuberculosis was quantitatively estimated in the lungs, spleen, and liver of the sacrificed pigs. A new method of establishing

numerical values corresponding as objectively as possible to the gravity of the experimental disease is described as well as the statistical assessment of the results with the chi-square test.

Massive and repeated doses of heat-killed BCG or of H37Rv residues prepared with a relatively rapid lipid extraction procedure afforded a significant degree of allergy and protection in guinea pigs. Massive doses of BCG residues submitted to a very prolonged process of lipid extraction conferred a low and irregular degree of allergy and retained only an insignificant power of protection. Relatively small doses of acetone-extracted BCG (residue I corresponding to a standard 60 mg. per ml. concentration of BCG) prepared by the same prolonged process of extraction seemed to possess an enhancing effect on the development of experimental tuberculosis, although sensitizing no more than 22 per cent of the animals to tuberculin.

A filtrate of an old BCG culture, as well as a borate extract (mostly of protein nature) of mechanically disrupted BCG bacillary bodies, far from immunizing the animals seemed to have enhanced their disease.

Sumario

Algunas Propiedades Biológicas de las Micobacterias Consecutivamente a la Extracción de Lípidos. La Facultad de Iniciar Resistencia Adquirida

Usando las técnicas de Macheboeuf y Anderson, se prepararon residuos bacilares de distinto contenido lípido por medio de la extracción solvente orgánica neutra del BCG y de las cepas H37Rv del *Mycobacterium tuberculosis*. Se inyectaron en cobayos suspensiones salinas de estas preparaciones. A fin de apreciar la capacidad de estos residuos para excitar resistencia activa, se utilizó un experimento de tipo de provocación-sacrificio. Se valuó cuantitativamente la tuberculosis macroscópica en los pulmones, bazos e hígados de los cobayos sacrificados. Se describe un nuevo método para establecer valores numéricos que corresponden lo más objetivamente posible a la gravedad de la enfermedad experimental, e igualmente la valoración estadística de los resultados con la prueba del cuadrado de la contingencia (χ^2).

Dosis masivas y repetidas de residuos de BCG o de H37Rv matados al calor, y preparados con un procedimiento de extraccefon relativamente rápida de lípidos suministran un grado importante de alergia y protección en los cobayos. Dosis masivas de residuos de BCG sometidos a un procedimiento muy prolongado de extracción de lípidos otorgan una alergia de forma baja e irregular y no retienen más que una facultad insignificante de protección. Dosis relativamente pequeñas de BCG extraído con acetona (residuo I que corresponde a una concentración normalizada de 60 mg. por ml. de BCG), preparado por el mismo procedimiento prolongado de extracción, parecen poseer un efecto elevador sobre la evolución de la tuberculosis experimental, aunque no sensibilizan a más de 22 porciento de los animales a la tuberculina.

Un filtrado de un cultivo viejo de BCG, así como un extracto boratado (principalmente de naturaleza proteica) de cuerpos bacilares desbaratados mecánicamente de BCG, en vez de inmunizar a los animales, parecen haber agudizado la enfermedad en éstos.

Resume

Certaines propriétés biologiques des mycobactéries après extraction des lipides

L'extraction des lipides des souches BCG et H37Rv de *Mycobacterium tuberculosis*, à l'aide de solvants organiques neutres et selon les techniques de Macheboeuf et de Anderson, a permis d'obtenir des résidus bacillaires de teneur lipidique variée. On a immunisé des cobayes à l'aide de suspensions aqueuses (sérum physiologique) de ces diverses préparations et on a appliqué le type d'expérience "infection-sacrifice" afin de vérifier si de tels résidus pouvaient induire une résistance active chez ces animaux. Les lésions tuberculeuses macroscopiques furent appréciées quantitativement sur les poumons, la rate et le foie des animaux sacrifiés. Une nouvelle méthode de traduire, aussi objectivement que possible, la gravité de l'invasion tuberculeuse expérimentale en valeurs numériques est décrite, de même que l'évaluation statistique des résultats à l'aide du test "Chi carré."

Des doses massives et répétées de BCG tué par la chaleur ou de résidus bacillaires préparés à partir de la souche H37Rv à l'aide d'un procédé relativement rapide d'extraction des lipides ont provoqué chez les cobayes des taux significatifs d'allergie et de protection. Des doses massives de résidus bacillaires préparés à partir de la souche BCG à l'aide d'une technique plutôt prolongée d'extraction des lipides n'induisirent chez les cobayes que des réactions irrégulières d'allergie ainsi qu'un degré non significatif de protection. Des doses relativement faibles de BCG extrait à l'acétone (Résidu I en concentration correspondant à 60 mg./ml. de BCG standard) et préparé selon la même technique prolongée d'extraction des lipides semblèrent aggraver la tuberculose

expérimentale des cobayes, quoique ne sensibilisant que 22% des animaux à la tuberculine.

Un filtrat d'une vieille culture de BCG ainsi qu'un extrait au borate (principalement de nature protéique) de BCG désintégré mécaniquement aggravèrent la maladie de nos animaux plutôt que de les immuniser.

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PREDNISONE IN THE TREATMENT OF TUBERCULOUS PLEURAL EFFUSIONS[1]

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Introduction

The corticosteroids and corticotropin in the therapy of tuberculosis still occupy a somewhat anomalous position. Only a few years ago their use or advocacy would certainly have met universal condemnation. At present, however, few clinicians would hesitate to employ them, along with appropriate antimicrobial therapy, in any of the following situations: (*1*) tuberculous meningitis, (*2*) fulminating acute pneumonic and miliary tuberculosis, (*3*) far advanced progressive pulmonary tuberculosis uncontrollable by antimicrobials alone, (*4*) drug hypersensitivity to a vital antituberculous agent, and (*5*) coexistence of a collagen or other disease in which use of these hormones is mandatory. Nevertheless, there is still little experience and much resistance to the use of these hormones in those forms of tuberculous disease in which some choice of therapy still remains, as in minimal pulmonary infiltrations and pleural effusions. This reluctance still lingers from the early 1950's when numerous reports were published describing the deleterious effects of these hormones on laboratory animals and a few patients with tuberculous infections. In neither of these situations, however, were the subjects protected by antimicrobials; nor, even in the absence of such protection, were these hormones unanimously considered destructive without some qualification.

On the debit side, cortisone and, to a lesser extent, corticotropin were found to influence tuberculous lesions adversely in the guinea pig (1), the naturally resistant albino rat (2, 3), the mouse (4, 5), and the rabbit (6, 7). However, in other experiments, no enhancement or exacerbation of the disease process could be demonstrated in the very susceptible guinea pig (8–11). No alteration in the nature of the tissue response or greater extent of the disease were found at autopsy (12). In the rabbit, increased phagocytic activity and less lymph node involvement were noted in spite of a greater number of tubercles and organisms (13).

Review of the Literature

The first tuberculous patients treated with cortisone and corticotropin consisted of thirteen patients who received this therapy for other diseases, but in whom tuberculosis was unsuspected or undiagnosed prior to treatment, and twenty other patients with known active pulmonary tuberculosis given these hormones deliberately. The former group all developed fulminating tuberculosis of various types; pulmonary in five cases (14–17), miliary in four cases (18–21), meningeal in two cases (22, 23), lymphadenitis in one (14), and osseous in one (23). This experience obviously had a very sobering effect on clinicians everywhere.

Nearly all of the twenty patients with known active pulmonary tuberculosis in the second group experienced partial or complete symptomatic improvement, but the majority showed various degrees of roentgenographic deterioration (24, 25, 28, 29). Five patients, however, showed no change, and in two who died no deleterious hormonal effect on the tuberculous disease could be demonstrated (26, 27). Two patients actually showed roentgenographic improvement (30).

Thus, while there is sufficient recorded experience interdicting the use of corticotropin or the adrenal corticosteroids alone in any form of tuberculosis, the entire problem is placed in a new perspective by the concomitant use of antimicrobial drugs. The early experiments with combined hormonal and antimicrobial treatment were confined to streptomycin and its dihydro derivative. Nevertheless, their value in counteracting cortisone-induced tuberculous spread, even in the highly susceptible guinea pig, was demonstrated in both effective and subeffective doses (1, 31). Although the question of the completeness of this protection arose (9), it was shown that, under certain conditions, animals treated with streptomycin and cortisone fared better than those treated with streptomycin alone (12). Similarly, corticotropin and dihydrostreptomycin produced better results in rabbits than dihydrostreptomycin alone (32). The albino rat was reported completely protected from cortisone by streptomycin (3). More recent

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rabbit experiments appear to prove conclusively that any adverse effects of the hormones can be nullified if used in conjunction with the antimicrobial drugs, corticotropin being the hormone of choice (34, 35).

Combined steroid-antimicrobial therapy first met general clinical acceptance in the treatment of tuberculous meningitis because of the desperate nature of such cases and because its use was rational (36, 37). Hormonal inhibition of granulation and fibrosis could be expected to be especially useful in maintaining a patent subarachnoid space and normal cerebrospinal fluid dynamics. Subsequent reports on the subject all stress steroid-antimicrobial therapy as the treatment of choice in tuberculous meningitis (38–43). In no case so treated has any dissemination of the tuberculous disease yet been reported, and this therapy is clearly superior to the earlier regimens involving intrathecal antimicrobials and debriding enzymes.

The early favorable experiences with the steroids in tuberculous meningitis were harbingers of their wider application to other forms of tuberculosis. Corticotropin was employed to combat hypersensitivity reactions to antimicrobials in cases of active tuberculosis (44). Not only was this hormone successful in suppressing such hypersensitivity in six of eight patients, but gastrointestinal intolerance to para-aminosalicylic acid (PAS) was relieved in five others. There was a coincidental clinical and roentgenographic improvement in all patients, even in those who had failed to respond to the antimicrobials alone.

Rapidly thereafter all forms of tuberculosis were investigated relative to their response to combined hormonal and antimicrobial therapy, with claims of cure or improvement in the majority of patients so treated. Of a series of 160 cases consisting of pleural effusions and parenchymal tuberculosis, good results were reported in all fifty-four cases of pleural effusion, cure in thirty-five cases of parenchymal tuberculosis, and improvement in thirty others 45). Both corticotropin and cortisone were used in this series. Corticotropin was given in amounts of 300 to 350 mg. weekly and cortisone in amounts of 50 mg. daily. Treatment was given in three-week courses and was accompanied by streptomycin and isoniazid administration. In another series of one hundred twenty cases better results were obtained with the concurrent use of hydrocortisone or prednisone and specific antituberculous drugs than with the latter alone in cases of acute pneumonic, miliary, and serous tuberculosis, as well as in meningitis (46). In a still more recent report, exudative parenchymal lesions, pleurisy, and tuberculous peritonitis are described as clearing more rapidly with than without hormonal therapy (47). One patient with active far advanced pulmonary tuberculosis requiring steroid therapy because of coexistent lupus erythematosus, and therefore treated with corticotropin, streptomycin, and PAS, experienced not only rapid remission of his collagen disease but an unusually rapid regression and inactivation of his tuberculosis as well (48). Enthusiasm for combined steroid-antimicrobial therapy appears to be growing.

Observations

At the Harlem Hospital the writers have used corticotropin and corticosteroids in association with antimicrobials for the past several years to treat cases falling into one or more of the five categories cited in the introduction of this paper. The results have been uniformly good, and the writers are convinced of the efficacy and safety of steroid therapy in tuberculosis, if accompanied by an adequate antimicrobial regimen. It was decided, therefore, to concentrate on the use of the hormones in pleural effusions, an area where sound theoretical indications for steroid therapy exist. Accepting that tuberculous pleurisy with effusion is a particularly hyperergic manifestation of tissue sensitivity to tuberculin (49), it follows that especial benefits could logically be expected from the anti-allergic action of corticotropin and the corticosteroids. Moreover, their anti-inflammatory properties should prevent the formation of organized pleural exudates, a particularly distressing sequel of long-standing effusions.

For the past twenty months 26 cases of tuberculous pleural effusions have been treated with steroids and antimicrobials. Only those patients were selected in whom no contraindication to steroid therapy existed. There were 15 males and 11 females in the series, and the age range was from twenty-six to fifty years. Thoracentesis on all of the patients yielded typical amber-colored fluid. Examination of cell blocks of the fluid of 14 of these patients disclosed an inflammatory exudate with lymphocytic preponderance and no evidence of neoplastic disease. Bronchoscopy of 5 of the patients showed no signs of malignant or other endobronchial lesions. Tubercle bacilli were cultured from the chest fluid of 4 patients and microscopy of the sputum was positive for acid-fast bacilli in one patient (figures 1 to 3). In 2 of the patients, the diagnosis of tuberculous effusion was made solely on clinical grounds. All of the patients had elevated temperatures on admission.

As soon as a diagnosis of pleural effusion was made, a thoracentesis was done and every effort

FIG. 1 (Upper). M. G., 38-year-old female, was admitted to the hospital on February 25, 1958, with massive right pleural effusion. Chest tap yielded 1,400 ml. of straw-colored fluid. Sputum was positive for acid-fast bacilli on microscopy on March 3, 1958.

FIG. 2 (Center). One month after admission roentgenogram revealed only residual pleural thickening at right base overshadowed by pendulous breast, but a parenchymal lesion is noted in the right first and second anterior interspaces. Patient left the hospital against advice on April 9, 1958, after having received a total of 320 mg. of prednisone, but continued receiving antimicrobial therapy in the clinic.

FIG. 3 (Lower). Roentgenogram four months after appearance of parenchymal lesion shows this to have completely resolved with no further significant change at right base. Sputum negative for *Mycobacterium tuberculosis*.

was made to remove all of the fluid possible. The patients were then placed on a regimen of 1 gm. of dihydrostreptomycin three times weekly and 300 mg. of isoniazid daily. This regimen is still being continued and will be maintained until a total of at least two years from the initiation of therapy has elapsed. Steroid therapy was started simultaneously.

The oral steroid prednisone (Meticorten®) was employed in all cases. The dosage schedule used was 30 mg. daily for four days, 20 mg. daily for seven to fourteen days (depending on response), then 10 mg. daily for the balance of a four-week period. One patient received treatment for six weeks. In no case was the hormone administered for more than six weeks nor was the total administration allowed to exceed 600 mg. during the entire course of treatment. Most patients received 25 units of corticotropin at the completion of the course of prednisone to restimulate endogenous adrenal activity. A program of partial bed rest was enforced for two to three weeks and then was gradually liberalized.

In no case was any hormone or other medication instilled intrapleurally and, with the exception of the single dose of corticotropin at the conclusion of prednisone therapy, this was also not used during the course of treatment. Intrapleural therapy was deliberately avoided in order to confine the study to the effects of prednisone and the antimicrobial drugs systemically. Prednisone was used instead of cortisone because of its established superiority with respect to lack of undesirable side reactions.

In all of the 26 patients there was a remarkable symptomatic response. Rapid defervescence, increased appetite, an enhanced sense of well-being, and greater physical vigor were noted within twenty-four to forty-eight hours of the start of hormone administration. These phenomena occurred even in the 3 patients admitted in an advanced stage of debilitation. In many instances the subjective improvement was so marked that the patients had to be admonished to desist from unnecessary physical activity. It cannot be alleged that this immediate clinical amelioration was due to the initial thoracentesis alone, since no evidence of improvement was

FIG. 4 (Upper). H. L., 40-year-old male, admitted to the hospital on January 8, 1957, with massive left pleural effusion. Admission thoracentesis yielded 800 ml. of straw-colored fluid and prednisone therapy was started on January 9, 1957. However, fluid reaccumulated and a chest tap was repeated on January 18, 1957, yielding an additional 1,000 ml. of fluid. Both specimens were positive for *Mycobacterium tuberculosis* on culture.

observed before the steroid therapy was instituted.

In 25 patients, follow-up roentgenographic examinations revealed that the small collection of fluid not removed by the thoracentesis cleared in three to six weeks, with a median of five weeks. This is in phenomenal contrast to previous experience in which residual effusion persisted up to six months in about 25 per cent of the cases, and, in a smaller percentage, persisted much longer, with ventilatory crippling secondary to fibrous organization of the residual exudates.

The amount of fluid present originally apparently had no bearing on the rapidity or totality of the response. Only one patient showed signs of sufficient reaccumulation to require a second thoracentesis shortly after the first, but following this his further response was satisfactory (figures 4 to 6). A minimal coexistent parenchymal lesion in another patient showed initial clearing in about six weeks and complete clearing in four months (see figures 1 to 3). The average hospital stay of these patients varied from three to twelve weeks, with a median of eight weeks. This again is in marked contrast to the pre-steroid era, when the average hospital stay on this service was at least six months.

In 12 of the series of 26 patients the clearing of the effusion was complete to the point of an entirely normal roentgenogram. In the remaining 14 cases, costophrenic blunting or a slight degree of basal pleural thickening remained as the only tell-tale evidence of pre-existing pathology. In none of these cases is there any indication that the clinical course will terminate in a disabling fibrothorax.

Fourteen of the 26 patients have now been followed for at least eighteen months, and in not a single one has there been any evidence of recrudescence of pleural disease or the appearance of any parenchymal lesions. It only remains to be seen what the clinical course will be after chemotherapy is discontinued. According

FIG. 5 (Center). Roentgenogram seventeen days after second chest tap shows a steady absorption of fluid with no evidence of reaccumulation. Mediastinal structures approaching normal position.

FIG. 6 (Lower). Roentgenogram more than one and one-half years later is entirely normal.

to present knowledge, however, it would seem unjustifiable to attribute to steroid therapy given for a few weeks two years previously any reactivation of tuberculous disease that remained healed for this entire period of time.

Summary

Corticotropin and the corticosteroids, in association with antimicrobial therapy, have a definite place in the treatment of certain types and certain complications of tuberculosis. Having used steroid-antimicrobial regimens in certain categories of tuberculosis and associated complications with good results, it was decided to employ such a regimen to treat tuberculous effusions, in which good theoretic indications for such therapy exist. Twenty-six patients were treated with dihydrostreptomycin, isoniazid, and prednisone, which were started immediately after an initial thoracentesis. The steroid was used in gradually decreasing doses down to a maintenance level, and was continued for four weeks—and in one case, six weeks. One dose of corticotropin was given at the termination of treatment. Intrapleural instillations were not used. All patients showed a remarkable symptomatic response in twenty-four to forty-eight hours.

With this regimen the fluid was found to clear in three to six weeks, with a median of five weeks. The average hospital stay ranged from three to twelve weeks, with a median of eight weeks. This is in marked contrast to previous experience on this hospital service.

Only one patient required a repeat thoracentesis. In another, a coexistent pulmonary lesion cleared completely in four months. Twelve of the 26 patients in this series showed roentgenographic clearing to the point of a normal film, and the remaining 14 showed minimal degrees of basal pleural thickening as a permanent stigma. Disabling fibrothorax has not been observed. On the basis of these results, it is believed that tuberculous pleural effusions constitute a definite added indication for the use of steroid therapy at this time.

Sumario

La Prednisona en el Tratamiento de los Derrames Pleurales Tuberculosos

La córticotropina y los córticosteroides, combinados con la terapéutica antimicrobiana, tienen su puesto bien definido en el tratamiento de ciertas formas y ciertas complicaciones de la tuberculosis. Habiendo usado con buenos resultados regímenes esteroido-antimicrobianos en ciertas clases de tuberculosis y de complicaciones vinculadas con éstas se decidió emplear un régimen de ese género para tratar los derrames tuberculosos, en los que existen buenas indicaciones teóricas para dicha terapéutica. A 26 enfermos se les trató con dihidroestreptomicina, isoniacida y prednisona, que se empezaron inmediatamente despues de una toracéntesis inicial. El esteroide se usó a dosis gradualmente menores hasta una cifra de sostén, continuándose cuatro semanas, y en un caso, seis semanas. Al terminar el tratamiento, se administró una dosis de córticotropina. No se usaron instilaciones intrapleurales. Todos los enfermos mostraron una notable respuesta sintomática en veinticuatro a cuarenta y ocho horas.

Se observó que con este régimen el líquido se despejaba en tres a seis semanas, con un promedio de cinco semanas. La estancia media en el hospital varió de tres a doce semanas, con un promedio de ocho semanas. Esto ofrece un contraste bien definido con lo observado antes en este servicio hospitalario.

Solamente un enfermo requirió otra toracéntesis. En otro, una lesión pulmonar coexistente se despejó totalmente en cuatro semanas. Doce de los 26 enfermos de esta serie mostraron un despejo radiográfico que culminó en una radiografía normal y los otros 14 revelanan mínimas proporciones de engrosamiento basal de la pleura como estigma permanente. No se observó fibrotórax incapacitante. A base de estos resultados, parece que los derrames pleurales tuberculosos constituyen una nueva indicación bien definida para el uso corriente de la esteroidoterapia.

Resume

La prednisone dans le traitemente de l'épanchement pleural tuberculeux

La corticothrophine et les corticostéroïdes, en association avec la thérapeutique antimicrobienne, occupent une place définie dans le traitement de certains types et de certaines complications de la tuberculose. Après avoir utilisé avec succès une thérapeutique stéroïdo-antibactérienne dans certaines catégories de tuberculose et de complications associées, il a été décidé d'utiliser cette médication pour traiter les épanchements tuberculeux, en présence d'indications théoriques favorables. Un traitement par la dihydrostreptomycine, l'isoniazide et la prednisone a été appliqué chez 26 malades immédiatement après une thoracocentèse initiale. Le stéroïde a été utilisé à doses graduellement décroissantes jusqu'à une dose

d'entretien et a été administré pendant quatre semaines et, dans un cas, pendant six semaines. Une dose de corticotrophine était donnée à la fin du traitement. On n'a pas eu recours aux instillations intrapleurales. Une réponse symptomatique remarquable a été observée chez tous les malades dans les vingt-quatre à quarante-huit heures.

Avec ce régime thérapeutique, le liquide s'est éclairci en trois à six semaines la moyenne étant de cinq semaines. Le séjour moyen à l'hôpital variait entre trois à douze semaines la moyenne étant de six semaines. Ceci contraste nettement avec l'expérience antérieure acquise dans ce service hospitalier.

La répétition de la thoracocentèse n'a été nécessaire que chez un seul malade. Chez un autre, une lésion pulmonaire coexistence a disparu complètement en quatre mois. Parmi les 26 malades de cette série les opacités radiologiques ont disparu chez 12 d'entre eux, a tel point qu'on a obtenu des clichés normaux, et les 14 autres n'ont conservé comme altération permanente qu'un faible degré d'épaississement pleural à la base. La sclérose thoracique invalidante n'a pas été observée. Sur la base de ces résultats, les auteurs estiment que les épanchements tuberculeux constituent à l'heure actuelle une indication supplémentaire définie de la stéroïdothérapie.

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A STUDY OF THE NATURE OF ARTERIAL OXYGEN LACK USING AN IMPROVED RAPID METHOD OF MEASURING OXYGEN TENSION[1, 2]

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Introduction

Knowledge of the partial pressure of oxygen in arterial blood (PaO_2) is of considerable importance to both the clinician and the physiologist under a variety of conditions.

Whereas the polarographic method has been known for many years, it was not until 1938 that Baumberger (1) and later Berggren (2) developed practical techniques for utilizing this method for determining PaO_2. A number of other electrode systems have been developed (3–6), but each had serious shortcomings. The current theoretic concepts of this method are presented by Connelly (7). The most recent adaptation of the polarograph to measure oxygen was devised by Clark (8), who invented an electrode system which separates the silver–silver chloride anode and platinum cathode cell from the solution being analyzed by a semipermeable membrane.

The characteristics of this electrode system were previously subjected to detailed study by the present writers (9). Similar systems were examined by Reeves and associates (10) and also by Kreuzer and co-workers (11). More recently, Severinghaus and Bradley (12) have designed an improved cuvette system and calibration method which is used with a specially designed portable polarograph amplifier and meter.

The principal advantage of the oxygen cathode-polarographic method is that it provides accurate rapidly reproducible measurements of the partial pressure of oxygen in solutions at all levels of oxygen concentration from 0 to 100 per cent, and is unaffected by changes in hematocrit, pH, and pCO_2 (9). The present writers and their associates further verified this method by comparisons with oxygen tensions determined by the Van Slyke-determined oxygen saturation and pH as well as the micro-bubble equilibration technique of Riley and co-workers (13). Actually, it is well known that the method of determining blood oxygen tension from Van Slyke-measured oxygen saturation and blood pH is not very accurate above pO_2 levels of 75 to 80 mm. of mercury (14) and the micro-bubble technique is not reliable above oxygen tension levels of 100 mm. of mercury. Therefore, it is apparent that none of the existing methods are truly satisfactory for comparison with the polarographic measurements which are calibrated by direct measurement of the diffusing current in the patient's blood equilibrated with gases of known oxygen tension.

It is the purpose of this presentation to describe improvements in the previously presented technique (9) and also to illustrate its value in the physiologic laboratory or at the bedside as an aid in the detection of hypoxia as well as the elucidation of its nature. Specifically illustrated are methods for determining the magnitude of veno-arterial shunts and for localizing arteriovenous shunts.

Materials and Methods

The methods employed, described in part previously (9), involve the use of indwelling vascular needles or catheters, and either an anaerobic collection of blood samples with immediate transmission of the sample from the collecting syringe to a silicone-coated cuvette into which the electrode is placed (figure 1), or by flowing the blood directly from the arterial needle via a short catheter into the cuvette. The cuvette is mounted in a constant temperature water or air bath at 37.5°C. A constant voltage of −0.6V is applied to the cathode while the blood is kept in motion past the potassium chloride-immersed electrode which is separated from the blood by a 0.004-inch polyethylene membrane. The rate of current flow in the electrode system at the applied voltage is directly proportional to the rate of reduction of oxygen at the cathode which, in turn, is dependent on the rate of diffusion of oxygen and the oxygen tension in the solution being examined (figure 2). The polyethylene membrane is permeable to gases

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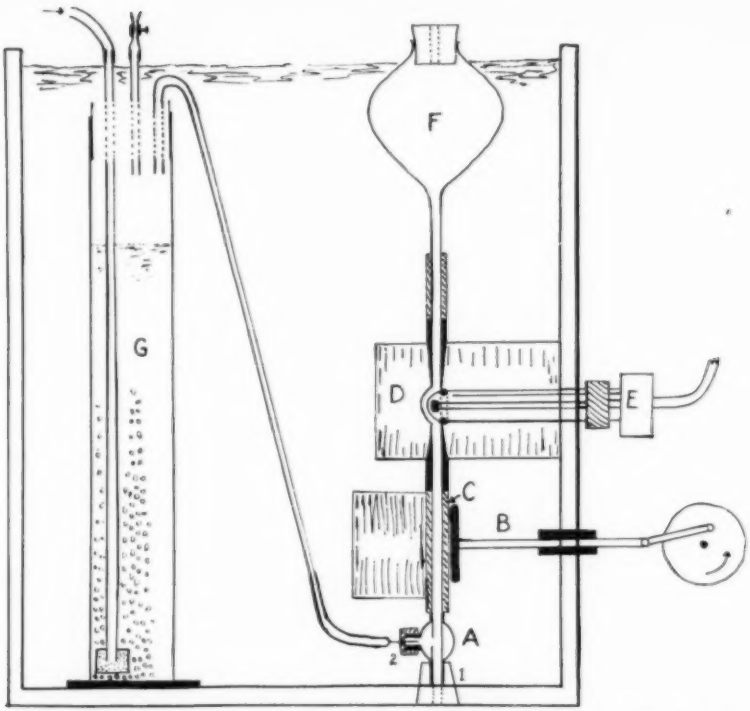

FIG. 1. A. Three-way stopcock for introducing the sample at "1" or the calibrating gases at "2." B. System for compressing short segment of rubber tubing. C. This is the method whereby the sample is kept in constant motion at the membrane. However, motion of the sample is no longer as imperative with a 0.004-inch membrane as with thinner membranes. The system is adjusted to compress the tubing at a rate of 1 to 2 times per second. D. Polystyrene cuvette for housing the oxygen cathode. E. The current is led to either standard or special (12) amperometric (polarographic) circuits. F. Small receptacle to allow breaking up of bubbles that form during the equilibration procedure. Equilibration with standard gases is accomplished in less than three minutes by flowing calibrating gas through A "2." One drop of caprylic alcohol added to the blood sample will usually prevent foaming. For more details on procedures, see references 9 and 12.

but not to other constituents of blood, which tended to inactivate other electrode systems in which the blood is in direct contact with the cathode.

The present system varies in two respects from the one previously described (9): First, a thicker membrane is used on the oxygen cathode. This has the advantage of diminishing the flow sensitivity to the point where, in most instances, it is not necessary to keep the blood in rapid motion. Second, by bubbling the standard gases directly through the vertical column of blood, equilibration occurs very rapidly and thus calibration becomes a greatly simplified procedure. The details of these modifications are being published elsewhere.

The patient's expired air is collected simultaneously and analyzed for carbon dioxide and oxygen so as to permit calculation of alveolar oxygen tension (PAO_2) by use of the alveolar equation:

$$PAO_2 = FIO_2 (B - BH_2O) - PaCO_2 \left(FIO_2 + \frac{1 - FIO_2}{R} \right) \quad (15).$$

Thus, by performing these studies at several levels of oxygen breathing including 100 per cent oxygen, the nature of alveolar air–arterial blood oxygen gradients (PAO_2–PaO_2) can be elucidated (2, 16).

Gradients arising from diffusion defects of the alveolocapillary membrane are exaggerated when subjects breathe low concentrations of oxygen, and tend to disappear on 100 per cent oxygen breathing.

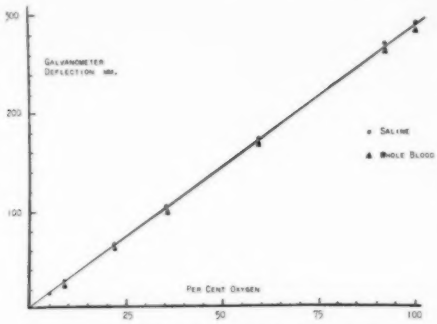

FIG. 2. This calibration line indicates that the readings for saline and whole blood are essentially the same, with a single cathode used in this system. In the system described previously (8), this was not generally true and, even with the present system, calibrations are not necessarily identical from one cathode to another. The reasons for this are not entirely clear to the writers, but would appear to be related in part to the amount of stretch placed on the membrane when it is put on the electrode tip. Thinner membranes give more divergent calibrations.

By knowing, and quite satisfactorily by assuming, the arterial-mixed venous oxygen difference, the magnitude of veno-arterial admixture can be estimated with the formula of Berggren (2):

$$100 - \frac{CaO_2 - C\bar{v}O_2}{(CaO_2 - C\bar{v}O_2) + G} = \text{per cent of systemic blood shunted,}$$

where $G = PAO_2 - PaO_2$ gradient times oxygen solubility coefficient of Sendroy 0.003 (with the patient on 100 per cent oxygen breathing).

The value obtained is based on the assumption that all the shunted blood is mixed venous blood not traversing capillaries of ventilated alveoli, and the shunt therefore might be intracardiac or intrapulmonary. The identification of right-to-left shunts as being intracardiac is dependent on other methods such as the well-known Evans blue (T-1824) dye techniques. On the other hand, most cardiac veno-arterial shunts are accompanied by arteriovenous shunting, so that with subjects breathing 100 per cent oxygen during blood sampling from the various chambers with the cardiac catheter a very precise method of detecting left-to-right shunting is obtained (17).

RESULTS

The first case is an example of the use of the 100 per cent oxygen breathing, polarographic oxygen method along with other techniques for the purpose of defining the cause of dyspnea in a patient who was ultimately demonstrated to have multiple pulmonary hemangiomatous fistulas.

Case 1. A. N. L., a 59-year-old dry goods merchant, who had smoked two and one half packages of cigarettes per day for thirty-five to forty years, was well until 1951, when he began to note moderate exertional dyspnea which was not progressive but was unrelenting. In 1953, he had an episode of severe left lower chest pain with leukocytosis and low-grade fever but, according to history, no evidence of roentgenographic or electrocardiographic abnormality to account for the symptoms. Early in 1957, fatigue and exertional dyspnea became more noticeable, so that cardiopulmonary evaluation was sought. Physical examination revealed an essentially healthy, middle-aged, white male with evidence of only a few sibilant rales in the right mid chest posteriorly which cleared with coughing. Vitalometer studies revealed essentially normal ventilatory function. The vital capacity was 4.3 liters or 110 per cent of predicted. The 0.5- and 1.0-second expiratory capacities were 60 and 75 per cent, respectively, of the vital capacity.

Thus there was no evidence of a ventilatory defect of any consequence. His spirogram exhibited two to three deep sighs per minute, and total minute ventilation was increased 11 liters per minute, which led to the suspicion that his dyspnea was psychogenic hyperventilation. However, in the course of routine evaluation of circulation times and exercise response, it was noted that oximeter readings with the patient supine revealed variation of oxygen saturation from 85 to 96 per cent, depending on the patient's level of breathing. Exercise oximeter values were normal. Both arm-to-ear and lung-to-ear circulation times were normal. Cardiac catheterization revealed normal pressures, and cardiac output (table 1) varied from 2.72 to 3.75 liters per square meter of body surface area depending on whether breathing was quiet or increased. The hemoglobin was 16.2 gm. per 100 ml., and the hematocrit was 50 per cent. Gas exchange studies are summarized in table 1. Like the oxygen saturation, the degree of shunt varied from 3 to 15 per cent, depending upon the level of ventilation.

The phenomenon of variation in the degree of shunting with changing respiratory levels has not, to the writers' knowledge, been previously reported. Although the exact mechanism is not known, this finding, which has been noted in similar cases, suggests a phasic respiratory change in resistance to blood flow either in the normal capillaries or the fistula which results in changes in the ventilation-blood flow relationship (14). Pulmonary angiography performed subsequently revealed bilateral pulmonary hemangiomas at the lung bases.

TABLE 1

PHYSIOLOGIC DATA ON GAS EXCHANGE IN A PATIENT WITH PULMONARY ARTERIOVENOUS SHUNTS (CASE 1)

	Room Air Breathing Normal Ventilation	Room Air Breathing Hyperventilaon	97 Per Cent Oxygen Normal Ventilation	97 Per Cent Oxygen Hyperventilation	97 Per Cent Oxygen Normal Ventilation
CO_2 content (mm. per L.)	16.65	15.56	15.60	14.00	17.00
O_2 saturation	88.0	96.9	100	100	100
pH	7.37	7.46	7.45	7.54	7.44
$PaCO_2$ (mm. Hg.)	34	27	32	21	31
Transfer gradient (alveolar pO_2-arterial pO_2) (mm. Hg.)	42	29	261	63	278
PaO_2 (mm. Hg.)	56	89	396	590	386
Ventilation (L. per min.)	8.0	13.8	9.3	19.0	7.62
Cardiac index (L. per min. per $M.^2$)	2.72	3.75	2.75	—	—

Case 2. E. B., a 41-year-old Negro female, was admitted with a history of dyspnea on exertion and orthopnea of five months' duration. On admission she was found to be in congestive heart failure with cardiomegaly and a circulation time of 25 seconds with a venous pressure of 22 cm. of water. She had hepatomegaly and, although physical examination of the chest was negative, a chest film showed a marked disseminated nodulation throughout both lung fields with a diffuse linear streaking, having the appearance of diffuse interstitial fibrosis. Serum globulin was slightly elevated and a liver biopsy was interpreted as granulomatous hepatitis, consistent with sarcoidosis.

Following adequate treatment of the congestive failure, which resulted in clinical compensation, dyspnea persisted and blood gas studies were performed (table 2).

Her subsequent course was slowly but progressively downhill and, when a post-mortem examination was performed two years after these blood gas studies, the pathologic diagnosis was that of sarcoidosis involving the heart, lungs, lymph nodes, liver, spleen, kidneys, bladder, and pituitary.

TABLE 2

PHYSIOLOGIC DATA ON GAS EXCHANGE IN A PATIENT WITH SARCOIDOSIS (CASE 2)

	Room Air Breathing	Low O_2 Breathing (17.5 Per Cent)	High O_2 Breathing (99.2 Per Cent)
O_2 saturation (per cent)	95	92	100
Arterial pCO_2 (mm. Hg.)	35	29	42
Arterial pH	7.39	7.42	7.32
Arterial pO_2 (mm. Hg.)	79	60	674
Alveolar pO_2 (mm. Hg.)	108	90	686
A-a gradient (mm. Hg.)	29	30	12

The negligible gradient obtained while pure oxygen was being breathed mitigates against pulmonary arteriovenous shunting of blood as a cause for the increased A-a gradient while breathing room air. The gradients of 29 and 30 mm. of mercury obtained when room air and low oxygen, respectively, were being breathed are compatible with an alveolar-capillary block to the diffusion of oxygen. The marked drop in pH and the rise in pCO_2 during 99 per cent oxygen breathing are suggestive of hypoventilation.

Case 3. J. H. J., a 36-year-old Negro male, was stabbed with an ice pick in the third left intercostal space parasternally. When seen in the hospital three hours after the accident, he was dyspneic and exhibited a holosystolic murmur and a thrill maximal over the site of the wound. In the next few days he showed evidence of heart failure with an increased venous pressure and circulation time as well as clinical and roentgenographic evidence of increasing heart size.

The cardiac failure eventually compensated; two weeks after admission he underwent a right-sided heart catheterization. Samples were withdrawn in the conventional manner from the various chambers for Van Slyke analysis while he breathed room air (table 3). The catheter was then reintroduced into the pulmonary artery and, after pure oxygen had been breathed for fifteen minutes, samples were once again drawn from various sites and analyzed polarographically (table 3).

This case illustrates a new and precise method of detecting the presence and location of arteriovenous shunting (17). Van Slyke values are often

TABLE 3

PHYSIOLOGIC DATA ON GAS EXCHANGE AND CIRCULATORY FUNCTION IN A PATIENT WITH VENTRICULAR SEPTAL DEFECT (CASE 3)

	Room Air Breathing O_2 Content	100 Per Cent O_2 Breathing pO_2	Pressure
	cc. per 100 cc.	*mm. Hg*	*mm. Hg*
Oxygen capacity	17.32	—	—
Pulmonary artery	14.29	85.2	61/18
High right ventricle	13.80	113.0	—
Low right ventricle	12.59	63.0	—
Mid right ventricle	—	93.0	69/6
High right atrium	10.11	45.4	—
Low right atrium	—	45.4	10
Superior vena cava	9.05	36.2	—
Brachial artery	15.74	337	103/67
Cardiac output	3.83 liters (systemic flow)		
Total pulmonary blood flow	8.23 liters		
Effective pulmonary blood flow	3.23 liters		
Right-to-left shunt	609 ml. or 15%		
Left-to-right shunt	4.40 liters		

confusing, but it is possible with the 100 per cent oxygen breathing–polarographic oxygen technique to accurately identify the site of arteriovenous admixture.

Case 4. T. S., a 51-year-old white male, was transferred to Parkland Memorial Hospital for dialysis on the artificial kidney for severe azotemia resulting from anuria. During a transurethral resection the prostatic capsule was ruptured, followed by a period of acute urinary retention. During a subsequent five-hour interval of shock, he received 1,750 ml. of blood and 1,000 ml. of saline.

Upon arrival at this hospital four days later, the patient was disoriented, and serum K^+ was 6.0. He exhibited no pulmonary difficulty at this time, and physical examination of the chest revealed only a few scattered rales in both bases. A six-hour hemodialysis was undertaken and the space of Retzius was drained of a large amount of urine, with subsequent clinical improvement. However, anuria persisted and azotemia was eventually progressive. Four days later, complaints of severe chest pain and breathlessness accompanied by harsh breath sounds and crackling rales, particularly evident in the left lung, appeared. A chest film showed mottled and confluent densities throughout both lung fields. Because of increasing intercostal retraction and respiratory rate while breathing oxygen unassisted, tracheostomy was performed to facilitate continuous assisted respiration by intermittent inspiratory positive pressure breathing. Interestingly enough, cyanosis was not apparent although the hemoglobin was only 8.2 gm. per 100 ml. Blood gas studies were performed following the tracheostomy (day 1, table 4).

The patient was maintained on continuous breathing assistance. It was found necessary to increase the positive pressure progressively to avoid obvious intercostal retraction and respiratory distress. Blood gas studies were repeated on the following morning (day 2, table 4). That evening, higher and higher pressure settings were required to prevent severe respiratory distress which was believed to be due to superimposed progressive pulmonary edema. An intravenous drip of Arfonad®,[3] 1 mg. per ml. of 5 per cent glucose in distilled water, was started, and titration of the blood pressure to 70 systolic, 50 diastolic in mm. of mercury resulted in a rapid decrease in the pressures required for effective assistance of ventilation. In the next two days, the pulmonary status appeared to improve clinically and blood studies corroborated this impression (days 3 and 4, table 4).

Obviously, a profound metabolic acidosis was present which was overcompensated while breathing on the inspiratory positive pressure breathing machine, in spite of the fact that 400 cc. of dead space was added to conserve carbon dioxide. Nevertheless, it was considered better to keep the patient slightly alkalotic in view of the severely elevated serum potassium, 7.4.

Because of the increasing anemia (the hemoglobin was now 4.5 gm. per 100 ml.), it was elected to transfuse 1,000 ml. of blood slowly the next day. Blood studies were again repeated (day 5, table 4).

The patient's course from this point was rapidly downhill, with unrelenting shock, unconsciousness, and death. Post-mortem examination revealed interstitial pneumonitis, "uremic pneumonitis," and mild hemorrhagic pulmonary edema. All tissues showed evidence of anoxic change.

This is an example of the use of this method for assaying the nature of the anoxic defect in a seriously ill patient. With the polarograph this information can be obtained at the bedside. This patient exhibited varying degrees of intrapulmonary veno-arterial admixture owing to extreme ventilation-blood flow distribution disturbances which characterize hemorrhagic pulmonary edema. The response to Arfonad obtained in this case is believed to be another example of the usefulness of this agent in certain cases of pulmonary edema (18).

[3] Arfonad is the registered trade name for D-3,4 - (1′,3′ - dibenzyl - 2′ - ketoimidazolids) - 1,2-trimethylene thiophanium D camphor sulfonate.

TABLE 4

PHYSIOLOGIC DATA ON GAS EXCHANGE IN A PATIENT WITH SEVERE INTERSTITIAL PNEUMONIA AND PULMONARY EDEMA (CASE 4)

	Day 1		Day 2		Day 3	Day 4		Day 5
	High O_2 Breathing Unassisted	IPPB/I–100 Per Cent O_2 15 Cm. H_2O Pressure*	IPPB/I–100 Per Cent O_2 15 Cm. H_2O Pressure	IPPB/I–100 Per Cent O_2 22 Cm. H_2O + 400 Cc. Added Dead Space	IPPB/I–100 Per Cent O_2 22 Cm. H_2O + 400 Cc. Added Dead Space	IPPB/I–100 Per Cent O_2 22 Cm. H_2O + 400 Cc. Added Dead Space	Room Air Breathing Unassisted	IPPB/I–100 Per Cent O_2 27 Cm. H_2O Pressure + 200 Cc. Added Dead Space
Respiratory rate	28	16	26	18	16	16	24	22
Arterial pO_2 (mm. Hg.)	62	195	65	310	425	520	45	75
Arterial pCO_2 (mm. Hg.)	36	28	21	29	33	20	29	15
Arterial pH	7.38	7.50	7.61	7.46	7.40	7.51	7.38	7.28
O_2 saturation (per cent)	90	100	96	100	100	100	78	92
A-a pO_2 gradient (mm. Hg.)	510	479	616	363	244	162	71	612
Estimated veno-arterial shunt†	—	27	31	22	16	11	—	34
Hemoglobin (gm. per 100 ml.)	8.2	—	—	—	—	4.5	—	—

* IPPB = intermittent inspiratory positive pressure breathing.

† Assuming the arteriovenous oxygen differences over the possible range of values based on previous determinations in similar cases. The average shunt is expressed as per cent of systemic blood flow. This applies in all subsequent tables.

Case 5. E. P., a 57-year-old white female, was hospitalized for complaints of dizziness, epigastric pain, facial floridness, and a tendency to bruise easily, all developing progressively over a two-year period. A diagnosis of polycythemia was established by hematologic studies which revealed a volume (radioactive chromium) of 7,705 ml. Blood gas studies are summarized in table 5. Pulmonary ventilatory studies indicated no significant derangement of mechanical function.

After four months, treatment with phenylhydrazine and radioactive phosphorus had reduced her hemoglobin to 15.4 gm. per 100 ml. with a hematocrit of 46. Blood studies were repeated as shown in table 5.

This case is typical of the results observed in a series of patients with polycythemia, both primary and secondary, who were studied before and after treatment (19). There was a direct correlation between the degree of polycythemia and the magnitude of the veno-arterial admixture. The mechanism of this apparent shunt is not clear at this time, but a number of mechanisms have been postulated. The apparent shunt may be a result of either "streaming effects" such as to permit an axial column of blood to pass without complete oxygenation, or extreme alterations in alveolar ventilation–pulmonary capillary blood flow relationships resulting from the opening of precapillary vascular channels, thus permitting venous blood to bypass the alveoli.

Case 6. J. A., a 36-year-old Negro female, had been hospitalized on a number of occasions with typical sickle cell crises over a period of fifteen years. She had received between two and three hundred blood transfusions. During an asymptomatic period, but when she was very anemic (the hemoglobin was 4.9 gm. per 100 ml.), blood gas studies were performed (table 6).

Vitalometry studies at this time revealed the 0.5-second expiratory capacity to be 1.3 liters or 72 per cent of the vital capacity, and the vital capacity, 1.8 liters or 37 per cent of predicted. Her total blood volume as measured by the radioactive chromium method was 5,360 ml. or 126 ml. per kg.

Her hemoglobin was raised to 12.3 gm. per 100 ml. by transfusion of 2,500 ml. of whole blood. Ventilatory tests failed to exhibit any change. Blood gas studies were repeated at this time (table 6).

The increase in alveolar-arterial pO_2 gradient after correction of the severe anemia in this patient was unexpected, but suggests the possibility of a mechanism similar to that operative in the polycythemic patient, *Case 5*. Since this patient's pulmonary vascular bed was effectively

TABLE 5

PHYSIOLOGIC DATA ON GAS EXCHANGE IN A PATIENT WITH POLYCYTHEMIA VERA (CASE 5)

	Before Treatment		After Treatment	
	Room Air Breathing	99 Per Cent O_2 Breathing	Room Air Breathing	99 Per Cent O_2 Breathing
Hemoglobin (gm. per ml.)	23.2	—	15.4	—
Hematocrit (per cent)	71	—	46	—
O_2 saturation (per cent)	92	100	96	100
Arterial pO_2 (mm. Hg.)	68	289	84	496
Arterial pCO_2 (mm. Hg.)	48	47	40	43
pH	7.34	7.35	7.36	7.36
A-a pO_2 gradient (mm. Hg.)	28	356	20	168
Estimated veno-arterial admixture (per cent)*	—	18.3	—	10.7
Ventilation (L. per minute)	7.68	7.52	13.00	11.22

* See † table 4.

TABLE 6

PHYSIOLOGIC DATA ON GAS EXCHANGE IN A PATIENT WITH SICKLE CELL ANEMIA (CASE 6)

	Before Transfusion			After Transfusion	
	Room Air Breathing	99 Per Cent O_2 Breathing	16 Per Cent O_2 Breathing	Room Air Breathing	99 Per Cent O_2 Breathing
Hemoglobin (gm. per ml.)	4.9	—	—	12.9	—
O_2 saturation (per cent)	90	100	79	79	95
Arterial pO_2 (mm. Hg.)	66	542	43	49	191
Arterial pCO_2 (mm. Hg.)	37	37	38	45	38
pH	7.36	7.40	7.38	7.34	7.41
A-a pO_2 gradient (mm. Hg.)	42	108	45	54	457
Estimated veno-arterial admixture (per cent)*	—	10.3	—	—	24.7
Physiologic dead space (cc.)	139	—	—	117	—

* See † table 4.

adjusted to the low hematocrit anemic state, and tended to respond to the high hematocrit as do the polycythemic patients, there may be some relationship between the hematocrit and pulmonary vascular resistance which tends to regulate pulmonary blood flow. The presence of veno-arterial admixture of this magnitude in patients with sickle cell anemia has been demonstrated in a series of twenty-one patients with sickle cell anemia previously studied and reported elsewhere (20), but once again the mechanism of the shunt is not clear.

SUMMARY

A convenient and accurate method for measuring oxygen tension by the use of the Clark oxygen polarographic cathode has been illustrated and described. Six cases are presented which serve to illustrate the value of this technique for the following: a rapid means of monitoring a critically ill patient's level of oxygenation and defining the nature of the anoxic disturbance; a method for identifying and quantitating right-to-left veno-arterial shunting; and a new and precise method for localizing central left-to-right arteriovenous shunts.

SUMARIO

Estudio de la Naturaleza de la Anoxia Arterial Usando un Método Rápido Perfeccionado para Medir la Tensión del Oxígeno

Descríbese e ilústrase un método conveniente y exacto para medir la tensión del oxígeno con el uso del cátodo polarográfico para oxígeno de Clark. Preséntanse 6 casos que sirven para demostrar el valor de esta técnica para lo siguiente: medio rápido de comprobar el grado de oxigenización de un enfermo en estado crítico y de definir la naturaleza del trastorno anóxico; método para identificar y medir la desviación venoarterial de derecha a izquierda; y método nuevo y preciso para localizar las desviaciones centrales de derecha a izquierda.

RESUME

Une étude de la nature de la déficience de l'oxygène artériel à l'aide d'une méthode perfectionnée rapide pour la détermination de la tension de l'oxygène.

Description et illustration d'une méthode commode et précise permettant de déterminer la tension de l'oxygène à l'aide de la cathode polaro-

graphique de Clark. Présentation de six cas servant à illustrer la valeur de cette technique qui constitue: un moyen rapide d'apprécier le niveau de l'oxygénation chez un malade dont l'état est critique et de définir la nature du trouble anoxique; une méthode pour identifier et évaluer le shunt veino-artériel de droite à gauche; et une nouvelle méthode précise pour localiser les shunts artérioveineux centraux de gauche à droite.

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SONIC FRAGILITY OF LEUKOCYTES FROM GUINEA PIGS VACCINATED WITH BCG[1, 2]

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Introduction

One of the most important basic problems in tuberculosis is the nature of acquired resistance. The various studies which have been made on the relative importance of humoral and cellular factors were critically reviewed in a recent publication by Youmans (1). While convincing evidence is still lacking, most investigators are reluctant to abandon completely the idea that some kind of antibody is involved. In the absence of such evidence, several workers have directed attention to the phagocytic cells. Suter (2) has reported that monocytes obtained from rabbits or guinea pigs vaccinated with BCG have the ability to inhibit the intracellular multiplication of tubercle bacilli *in vitro*. The ability of the monocytes to inhibit bacillary proliferation was the same whether serum from normal or vaccinated animals was used in the medium. Moreover, the serum of vaccinated animals did not inhibit multiplication of tubercle bacilli within monocytes obtained from normal animals. Investigations by Mackaness (3), however, revealed no significant difference in the growth rates of intracellular tubercle bacilli in monocytes from normal rabbits and from rabbits vaccinated with H37Rv or BCG. Various theories have been offered in explanation for this discrepancy of findings (3, 4).

More recently the possibility that a combination of humoral and cellular factors might prevail in acquired immunity to tuberculosis was reported by Fong and associates (5). Using techniques designed specifically to maintain constant monocytic populations, those writers observed that virulent tubercle bacilli (H37Rv) induced degeneration of both normal and immune monocytes (from rabbits vaccinated with BCG) cultivated *in vitro* in the presence of normal rabbit serum. In contrast, the serum of rabbits vaccinated with BCG protected immune but not normal cells against the degenerative effects of H37Rv. The protection of immune cells by heterologous antisera (anti-Salmonella and anti-ovalbumin) as well as by homologous antiserum (anti-BCG) against the degenerative effect of H37Rv appeared to establish that the serum factor was nonspecific (6). The ability of monocytes from animals immunized with BCG and the failure of monocytes of animals immunized with Salmonella to withstand degeneration by H37Rv, when both types of cells were cultivated in immune (anti-BCG) serum, indicated that the cellular factor was specific. Subsequent work, however, revealed a nonspecific element when it was found that vaccination of rabbits with BCG or with Brucella vaccine protected their monocytes against degeneration by both H37Rv and Brucella organisms when studied by the monocytic culture method (7).

These results demonstrated a cross-immunity in the ability of monocytes to resist destruction by virulent bacteria which might conceivably extend to any bacterial species which is typically intracellular in its parasitism, but not to primarily extracellular parasites. The present investigation was designed to determine whether the phenomenon might be extended even further, i.e., to disruption by mechanical trauma entirely divorced from bacteria or their derivatives. The results indicate that a situation similar to that described by Fong and associates prevails when monocytes are exposed to sonic vibration. In addition, experiments will be described which were designed to determine the relationship between sonic fragility and *in vitro* tuberculin cytolysis.

Materials and Methods

Vaccination: In preliminary studies, male, albino guinea pigs were vaccinated by the intramuscular injection of 1 ml. of paraffin oil containing 5 mg. (dry weight) of heat-killed human tubercle bacilli, H37Rv.[3] In all subsequent work

[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D. C.

[2] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 20, 1958.

[3] Culture originally supplied by Dr. William Steenken, Jr., Saranac Lake, New York.

the animals were vaccinated with BCG.[4] For this purpose, a portion of the pellicle from a nineteen-day-old culture on a modified Proskauer and Beck liquid medium (8) was ground with glass beads in sterile saline and standardized to contain 10 mg. per ml. according to the technique described by Youmans and Karlson (8). Male albino guinea pigs were inoculated intradermally with 0.1 ml. (1 mg.) of this bacterial suspension. Control animals received 0.1 ml. of sterile saline.

Collection of monocytes and serum: Normal and immune monocytes[5] were obtained from appropriate guinea pigs by the intraperitoneal injection of 30 ml. of sterile paraffin oil. Four days later the animals were sacrificed and the peritoneal cavity was washed with 30 ml. of Ringers solution containing 0.005 per cent heparin. The washings were transferred to a separatory funnel, and the oil was allowed to separate. The aqueous layer was drawn into a centrifuge tube and the oil layer was washed with 30 ml. of the heparinized Ringers solution. The aqueous layer was then drawn into a second centrifuge tube. The two tubes were centrifuged at 1,500 r.p.m. for fifteen minutes, and the cell butts were pooled in one tube in 10 ml. of Ringers solution by gently drawing the suspension up and down in a pipette. Cell counts were made with a Spencer hemacytometer, and each suspension was adjusted to contain approximately 5,000 cells per cu. mm. by appropriate dilution with Ringers solution. Differential smears of exudate cells obtained by this technique revealed approximately 80 to 90 per cent mononuclear cells. The cell suspensions were free of clumps and there appeared to be no consistent difference in the number of cells derived from normal and immune animals.

In most cases, the same animals which were used as sources of normal and immune monocytes served as donors of normal and immune serum.

Sonic fragility tests: One and five-tenth milliliter aliquots of normal or immune monocytic suspension were transferred to conical tip centrifuge tubes and centrifuged at 1,500 r.p.m. for fifteen minutes. After the supernates had been removed carefully with a pipette and discarded, the cell butts were resuspended in 1.5 ml. of test serum and the tubes were placed in a 37°C. water bath for thirty minutes. The cell-serum mixtures were then transferred to conical tip, polyethylene centrifuge tubes. Approximately 40 ml. of water were placed in the vibrator cup of a 50-watt, 9-kilocycle Raytheon magnetostriction oscillator, and the tubes were immersed in the water so that the level of fluid inside the tubes was the same as the water level on the outside. The temperature of the effluent cooling water was maintained at approximately 15°C. throughout the treatment period (three minutes at 75 volts). Cell counts were made before and after sonic treatment and the results were recorded as the per cent of cells destroyed.

Tuberculin cytolysis tests: In some of the studies the ability of serum samples to render normal monocytes susceptible to lysis by tuberculin *in vitro* was determined by a technique patterned after that of Miller and Favour (9). Monocytes were collected from normal guinea pigs by the method already described. The total leukocyte count was determined. A sample of sufficient size to contain approximately 75 million cells was centrifuged at 1,500 r.p.m. for fifteen minutes and the cell butt was resuspended in 3 ml. of freshly collected normal guinea pig serum. A 0.3 ml. sample of each test serum and 0.1 ml. of the normal monocytic suspension were transferred to each of two siliconized serologic tubes. To one tube 0.1 ml. of 0.9 per cent NaCl was added; to the other was added 0.1 ml. (5 γ) of PPD (Sharp and Dohme) dissolved in 0.9 per cent NaCl. The contents of the tubes were well mixed and total leukocyte counts were made. The tubes were then placed in a 37°C. water bath and mechanically shaken at a constant rate for two hours, after which a second leukocyte count was made. The PPD decrement was calculated as the per cent change in the tuberculin tube minus the per cent change in the saline control tube.

Results

In a preliminary experiment, monocytes and serum were obtained from 4 normal guinea pigs and from 4 animals which had been vaccinated two months previously with heat-killed tubercle bacilli in paraffin oil. Normal and immune cells were suspended in either normal or immune serum, and the susceptibility of the cells to disruption by sonic vibration was determined.

The results are presented in table 1. It is evident that immune monocytes suspended in immune serum were significantly more resistant ($P < 0.01$) to sonic disruption than similar cells in normal serum. Suspending normal cells in immune serum did not increase ($P > 0.1$) their resistance to sonic disruption. This experiment was repeated using another group of 4 normal and 4 experimental animals, eleven weeks post vaccination, with essentially the same results.

In view of these findings, it seemed of interest to determine whether vaccination of guinea pigs with viable bacilli (BCG) would similarly increase the resistance of their monocytes to sonic disruption. Also, it was thought pertinent to determine how soon after vaccination the phenomenon first became detectable.

[4] Obtained from Dr. S. R. Rosenthal, Tice Laboratory, University of Illinois, Chicago, Illinois.

[5] Throughout this paper the terms "normal" and "immune" will refer to cells or serum obtained from nonvaccinated and vaccinated animals, respectively.

TABLE 1

EFFECT OF NORMAL AND IMMUNE SERUM ON THE SONIC FRAGILITY OF NORMAL AND IMMUNE MONOCYTES

Type of Cell	Type of Serum	Mean Per Cent of Cells Destroyed by Sonic Vibration
Normal	+ Normal	40.0
Normal	+ Immune	38.5
Immune	+ Normal	30.1
Immune	+ Immune	16.2

Fifteen guinea pigs were inoculated with BCG and another group of 15 animals received an equal volume of saline. Groups of 3 vaccinated and 3 control guinea pigs were then used as sources of cells and serum according to the following scheme: The first 3 animals in each group were injected with paraffin oil the day following vaccination, and the others at five, eight, twelve, and fifteen days following vaccination. Monocytes and serum were collected four days after injection with paraffin oil, that is, five, nine, twelve, sixteen, and nineteen days after vaccination. Normal and immune cells were suspended in homologous serum and tested for sonic fragility in the usual fashion.

The results (table 2) show that, with immune monocytes, marked resistance to sonic disruption was seen in cells collected twelve days after vaccination or later. The sonic fragility of control monocytes remained fairly uniform throughout the experimental period. It should perhaps be re-emphasized that, contrary to the experience of Suter (2), no consistent difference was observed in the number of monocytes present in exudates from vaccinated and control animals.

The work of Fong and associates (6), referred to earlier, pointed out a basic difference in the effect of H37Rv and PPD on immune and normal monocytes. Immune, but not normal, cells were completely protected against the virulent tubercle bacilli when immune serum was present. In contrast, PPD had its greatest effect upon immune cells regardless of the type of serum in the medium. It thus appeared that it would be of value to determine the relationship, if any, between sonic fragility and the cytotoxicity of tuberculin. Two final experiments were designed to explore this possibility. The techniques used were based on those employed by Favour and associates (9, 10) in studies on the "plasma factor" responsible for tuberculin cytolysis *in vitro*.

In the first experiment monocytes were obtained from 3 normal guinea pigs and from 3 animals which had received BCG nineteen days previously. The cells were washed three times with 0.85 per cent NaCl containing 200 mg. per cent glucose and were resuspended in sufficient freshly collected normal guinea pig serum[6] to give a cell count of approximately 12,000 per cu. mm. The tubes were then mechanically shaken at a constant rate in a 37°C. water bath for six hours and were finally centrifuged at 16,000 r.p.m. for twenty minutes. Freshly collected normal guinea pig monocytes were added to duplicate samples of the individual serum supernates to give a concentration of approximately 5,000 cells per cu. mm. One set was used for sonic fragility tests; the other set was used to determine the susceptibility of the cells to *in vitro* cytolysis by PPD.

TABLE 2

SONIC FRAGILITY OF GUINEA PIG MONOCYTES AT VARIOUS INTERVALS AFTER VACCINATION WITH BCG

	Mean Per Cent of Cells Destroyed by Sonic Vibration				
	Days After Vaccination				
	5	9	12	16	19
Controls	29.1	25.3	29.5	31.7	28.1
Vaccinated	21.4	17.2	7.0	8.3	6.9

It may be seen from the results presented in table 3 that, when thoroughly washed immune monocytes are suspended in normal serum and incubated for six hours, a factor is released into the serum capable of (*a*) protecting normal monocytes against sonic disruption and (*b*) rendering normal monocytes susceptible to lysis by PPD.

The results of this study suggested that susceptibility to cytolysis by PPD confers upon cells a certain degree of resistance to sonic disruption. A final experiment demonstrated, however, that this is not necessarily true. Four normal guinea pigs and 4 animals which had been vaccinated fourteen days previously with BCG were injected intradermally with 5 γ of PPD. At the end of forty-eight hours, when the skin reactions in the vaccinated group measured 10 to 14 mm. induration, all 8 animals were bled. Freshly collected normal guinea pig monocytes were added to each serum sample. Sonic fragility tests and tuberculin cytolysis tests were performed in the usual fashion.

The results of this study are shown in table 4, in which it will be seen that, when normal mono-

[6] Containing 100 mg. per cent glucose, 10 units of potassium penicillin G, and 50 γ of streptomycin base per ml.

TABLE 3

SONIC FRAGILITY AND PPD CYTOLYSIS OF NORMAL MONOCYTES IN PRESENCE OF SERUM SUPERNATE FROM NORMAL AND IMMUNE CELLS

Type of Cell	Type of Serum Supernate*	Mean Per Cent of Cells Destroyed by Sonic Vibration	Mean PPD Decrement
Normal	+ Normal cells	22.0	+0.5
Normal	+ Immune cells	5.1	−16.1

* After incubation of appropriate cells in normal guinea pig serum for 6 hours at 37°C.

TABLE 4

SONIC FRAGILITY AND PPD CYTOLYSIS OF NORMAL GUINEA PIG MONOCYTES IN SERUM OF BCG-VACCINATED GUINEA PIGS

Type of Cell	Type of Serum*	Mean Per Cent of Cells Destroyed by Sonic Vibration	Mean PPD Decrement
Normal	+ Normal	44.0	−2.1
Normal	+ BCG	38.7	−24.2

* Collected 48 hours after a tuberculin skin test.

cytes are suspended in serum collected from BCG-vaccinated guinea pigs forty-eight hours after a tuberculin skin test, the cells are rendered susceptible to lysis by PPD. However, such cells are no more resistant to sonic disruption than are similar cells suspended in normal serum. This experiment was repeated, using another group of 4 normal and 4 experimental animals twenty-one days post vaccination, with similar results.

DISCUSSION

The experimental findings reported here provide rather convincing evidence that vaccination of guinea pigs with BCG confers upon their monocytes the ability to resist disruption by sonic vibration. It is interesting to compare the results of the present studies with those of workers who have used tissue culture methods to investigate the tubercle bacillus-monocyte relationship in acquired immunity. Fong and associates (5), as mentioned earlier, found that virulent tubercle bacilli (H37Rv) induce degeneration of monocytes from both normal and immune (BCG-vaccinated) rabbits *in vitro* in the presence of normal serum. In contrast, immune serum protected immune but not normal cells against the degenerative effect of the bacilli. The results of the present study, using guinea pigs vaccinated with heat-killed tubercle bacilli in paraffin oil, show that a similar situation prevails when the monocyte-serum mixtures are exposed to sonic vibration. Thus it would appear that both phenomena, resistance to degeneration by H37Rv and resistance to sonic disruption, depend somehow on the interaction of a humoral and a cellular factor. It is tempting to believe that the cellular factor might be related in some manner to the lytic effect of tuberculin on leukocytes of the tuberculin-sensitive host (11). An analysis of this possibility was carried out by Fong and associates (6), and it was their contention that monocytic degeneration by H37Rv and by tuberculin were independent phenomena. The results of the present investigation indicate that guinea pig monocytes which are susceptible to lysis by PPD may or may not demonstrate increased resistance to disruption by sonic vibration, depending on the experimental conditions used. To conclude, however, that the two phenomena are unrelated would seem to be premature without further investigation.

In the present experiments, sonic resistance of guinea pig monocytes was first detected twelve days after vaccination with BCG. Using a tissue culture technique, Suter (2) noted that the first evidence of inhibition of intracellular growth of tubercle bacilli occurred in cultures of monocytes which had been collected eight to fifteen days after vaccination at approximately the same time when positive tuberculin skin tests developed. The role of tuberculin skin sensitivity in the induction in monocytes of resistance to sonic disruption is yet to be determined.

Dietz and Mense (12), using citrated whole blood, measured the sonic fragility of leukocytes from normal subjects and from a group of tuberculous patients. In most patients the cells showed an increased resistance to sonic disruption. In a small group of patients, however, the leukocytes showed less resistance than normal. In the latter cases, therapy with amithiozone caused the resistance to increase toward normal.

No attempt seems justified at present to judge the importance of the ability of monocytes from BCG-vaccinated animals to resist disruption by sonic vibration. There could only be speculation as to its possible value in understanding the mechanism of acquired immunity. Nevertheless,

the phenomenon appears to be worthy of further investigation.

Summary

Immune monocytes (from guinea pigs vaccinated with heat-killed tubercle bacilli in paraffin oil) suspended in immune serum were significantly more resistant to disruption by sonic vibration than similar cells in normal serum. Suspending normal cells in immune serum did not increase their resistance to sonic disruption.

Marked resistance to sonic disruption was first detected in monocytes of guinea pigs vaccinated with BCG collected twelve days after vaccination.

When thoroughly washed immune monocytes were suspended in normal serum and incubated for six hours, a factor was released into the serum capable of protecting normal monocytes against sonic disruption and of rendering normal monocytes susceptible to lysis by PPD.

When normal monocytes were suspended in serum collected from BCG-vaccinated guinea pigs forty-eight hours after a tuberculin skin test, the cells were rendered susceptible to lysis by PPD. However, the cells were no more resistant to sonic disruption than were similar cells suspended in normal serum.

Sumario

Fragilidad Sónica de los Leucocitos de Cobayos Vacunados con BCG

Los leucocitos inmunes (procedentes de cobayos vacunados con bacilos tuberculosos matados al calor, en aceite de parafina) suspendidos en suero inmune fueron decididamente más resistentes a la disgregación por vibración sónica que las células semejantes en suero normal. La suspensión de células normales en suero inmune no acrecentó su resistencia a la disgregación sónica.

La existencia de notable resistencia a la disgregación sónica se descubrió primero en los monocitos de cobayos vacunados con BCG, recogidos a los doce días de la vacunación.

Cuando se suspendieron en suero normal monocitos inmunes y perfectamente lavados y se incubaron seis horas, se desprendió al suero un factor capaz de proteger a los monocitos normales contra la disgregación sónica y de susceptibilizarlos a la lisis por PPD (DPP).

Cuando se suspendieron monocitos normales en suero colectado de cobayos vacunados con BCG a las cuarenta y ocho horas de ejecutar una cutirreacción con tuberculina, se susceptibilizaron los glóbulos a la lisis por PPD. Sin embargo, los glóbulos no se mostraron más resistentes a la disgregación sónica que glóbulos semejantes suspendidos en suero normal.

Resume

Fragilité aux vibrations sonores des leucocytes de cobayes vaccinés par le BCG

Les monocytes immuns (de cobayes immunisés avec un vaccin à l'huile de paraffine contenant des bacilles tuberculeux tués par la chaleur) mis en suspension dans du sérum immun, étaient notablement plus résistants à la fragmentation induite par les vibrations sonores que les cellules similaires dans un sérum normal. La suspension des cellules normales dans un sérum immun n'augmentait pas leur résistance à la fragmentation par les ondes sonores.

Une résistance prononcée à la fragmentation par les ondes sonores a d'abord été décelée dans les monocytes de cobayes vaccinés par le BCG et recueillis douze jours après la vaccination. Quand des monocytes immuns, minutieusement lavés, étaient mis en suspension dans du sérum normal et placés en étuve pendant six heures, un facteur capable de protéger les monocytes normaux contre la fragmentation par les vibrations sonores et de sensibiliser à la lyse par le PPD les monocytes normaux, était mis en liberté dans le sérum.

Quand des monocytes normaux étaient mis en suspension dans un sérum recueilli chez les cobayes vaccinés par le BCG quarante-huit heures après une cutiréaction à la tuberculine, les cellules étaient sensibilisées à la lyse par le PPD. Toutefois, les cellules ne devenaient pas plus résistantes à la fragmentation par les ondes sonores que des cellules semblables en suspension dans le sérum normal.

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INVESTIGATION OF THE INFLUENCE OF ANTIMICROBIAL DRUGS ON THE ALLERGIC REACTION TO REINFECTION TUBERCULOSIS IN RABBITS[1,2,3]

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INTRODUCTION

In a previous paper (1), the influence of an antituberculous agent on the allergic reaction to a reinfection tuberculous infection in rabbits was described. The organisms used in these experiments were a living strain of *Mycobacterium tuberculosis* which had not previously been exposed to the agent. The purpose of the present report is to describe the influence of prior exposure of the organism to antimicrobial drugs and unfavorable environment on the host response of the living animal.

The growth of Mycobacteria *in vitro* is inhibited by low concentrations of certain therapeutic chemicals. In higher concentrations, for example, 1 mg. of isoniazid per ml., the compounds are lethal. It has been reported by Lewandowsky (2) that reinfection with heat-killed tubercle bacilli provokes the same reaction as that with living bacteria. According to Schwartz (3), Oppenheimer (4) and Yamamura and associates (5), instillation of extracts of tubercle bacilli will cause a reaction in the lungs resembling that obtained with heat-killed bacteria. Whether the same principle holds for drug-killed organisms is unknown. To resolve this question the following experiment was performed.

METHOD

Fifty-five adult albino rabbits were each infected with 1 mg. of tubercle bacilli of strain K-3 which proved to be susceptible to streptomycin, PAS, and isoniazid. The organisms were freshly recovered from the sputum of a patient and were injected subcutaneously into the right paravertebral region of the rabbits. The animals subsequently developed local tuberculous lesions and the tuberculin reaction became positive. After five weeks the animals were divided into seven groups and were subsequently treated in the following manner:

Group A consisted of 6 rabbits which were reinfected by intratracheal instillation into one lung with a 5-mg. inoculum of PAS-treated tubercle bacilli. The inoculum was prepared as follows: Cultures of strain K-3 were harvested and suspended homogenously in a 10 per cent solution of PAS. This solution was then incubated at 37°C. for six days. After six days the suspensions were centrifuged and washed twice with a saline solution. They were then suspended in physiologic saline in a concentration equal to 1 mg. per ml. Subcultures of these treated organisms yielded two hundred typical colonies after six weeks of incubation. A control culture of an untreated strain of K-3 tubercle bacilli showed numerous colonies after two weeks (and a confluent growth after six weeks) of incubation. The organisms of both cultures were morphologically typical. Both cultures proved to be susceptible to PAS.

Group B consisted of 10 animals which were reinfected with 5 mg. of heat-killed tubercle bacilli. The suspensions were prepared in the following manner: Cultures of strain K-3 were collected and suspended in physiologic saline. They were then heated in a Koch boiler at 98°C. for thirty minutes. After treatment the suspensions were incubated at 37°C. for six days and were then centrifuged and washed as before. The inoculum was prepared to contain 1 mg. per ml. The subcultures of these organisms yielded only a few colonies after eight weeks of incubation. Smears of the material disclosed countless morphologically typical organisms.

Group C consisted of 5 animals which were reinfected with 5 mg. of tubercle bacilli prepared in distilled water. Cultures of strain K-3 were harvested as before, except that distilled water was used instead of saline. The suspensions were then incubated at 37°C. for six days, collected, centrifuged, and washed twice. The final inoculum was suspended in physiologic saline. Subcultures of this material yielded numerous colonies after two weeks of incubation.

Group D consisted of 5 animals which were reinfected with 5 mg. of *Mycobacterium tuberculosis*, strain K-3. The inoculum was prepared by incubation in 0.2 per cent of isoniazid solution similar to the method of *Group A*. Subcultures of this material yielded no colony.

[1] From the Shyoraiso Sanatorium of Health Insurance, Chiba City, Japan.

[2] Present name: Shyoraiso Hospital of Social Insurance.

[3] Presented in part at the Fifty-third, Fifty-fourth, and Fifty-fifth annual meetings of the Japanese Society of Internal Medicine in 1956, 1957, and 1958, respectively.

TABLE 1

COMPARISON OF HYPERSENSITIVITIES BETWEEN THE DIFFERENT GROUPS OF ANIMALS REINFECTED BY DIFFERENT INOCULA

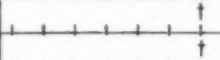
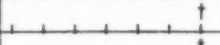
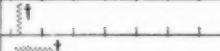
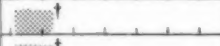
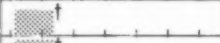
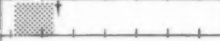
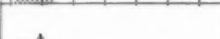
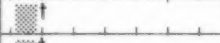
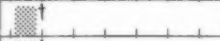
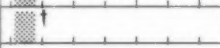
Group	Number of Rabbits	Roentgenographic Shadow (0 · 10 days · 20 days · 1 month · 40 days · 50 days · 2 months)	Extent of Lesion	Results of Microscopic Examination: Cell Infiltration	Exudative Lesion	Productive Lesion	Cavity	Scar	Number of Tubercle Bacilli	Colonies of Tubercle Bacilli
A.	RP-1		−			+		+	−	0
	RP-2		±					+	−	0
	RP-3		+				+		++	0
	RP-4		+			+		+	+	2
	RP-5		+			++		+	++	500
	RP-6		++			+		+	+	4
B.	RT-1		−	+				+	−	0
	RT-2		+	+		+		+	+	0
	RT-3		−					+	−	0
	RT-4		++			+++		++	++	0
	RT-5		+					+	−	0
	RT-6		++			+		+++	+	0
	RT-7*		+++	+++					+	
	RT-8*		++++	++++		+++			+	
	RT-9		++++	++++					−	
	RT-10		++++	++++					+	
C.	RW-1		+++	+++					+	420
	RW-2		+++	+++					+	2,100
	RW-3		++++	++++					+	
	RW-4		++++	++++					+	
	RW-5		++++	++++					+	
D.	RI-1		−	±					+	0
	RI-2		+	+					+	0
	RI-3		+	+					−	0
	RI-4		+	++					+	0
	RI-5		+	++					+	0

TABLE 1—*Concluded*

Group	Number of Rabbits	Roentgenographic Shadow (0 · 10 days · 20 days · 1 month · 40 days · 50 days · 2 months)	Extent of Lesion	Results of Microscopic Examination: Cell Infiltration	Exudative Lesion	Productive Lesion	Cavity	Scar	Number of Tubercle Bacilli	Colonies of Tubercle Bacilli
E.	RWA-1		+	+					+++	175
	RWA-2		+	+					+++	5
	RWA-3		++	++					++	35
	RWA-4		++	++		+			++	3,000
	RWA-5		++	++		+			++	0
F.	RS-1		++	++						1
	RS-2		++	++						
	RS-3		+++	+++						0
	RS-4		++	++						0
	RS-5		++++	++++						
	RS-6		+++	+++						
	RS-7		+++	+++						
	RS-8		++	++						
G.	R-1		+++	+++					++	2,000
	R-2		+++	+++					++++	2,880
	R-3		+++	+++	++				++	575
	R-4		++++	++++	+				−	
	R-5		+	+					−	12
	R-6		+	+		+			++	800
	R-7		+	+		+		++	−	0
	RA-1		++++	++++	+				+	
	RA-2		++	++	+				+++	
	RA-3		+	+		+			−	0
H.	P-1		++++	+	++++		++		++++	200,000
	P-2		++++	+	++++		++		++++	200,000
	PA-1		++	++		+		+	+++	500
	PA-2		++++	+++	++		+		++	20,000
	PP-1		++	++	++				++	550
	PW-1		+++	+	+	++			+++	5,600

* RT-7, RT-8. The heat-killed tubercle bacilli of those experiments were treated with distilled water in a manner similar to *Group C*.

† Killed.

Group E consisted of 5 animals which were reinfected with 5-mg. doses of resistant strain A-1. This strain was recovered from the sputum of a different patient and proved to be moderately resistant to streptomycin and PAS although susceptible to isoniazid. The organisms of this experiment were treated with distilled water in a manner similar to those of *Group C* above. Subcultures of this material yielded a few colonies after six weeks of incubation.

Group F consisted of 8 animals which were reinfected with 5-mg. doses of *Mycobacterium tuberculosis*, strain K-3. The inoculum was prepared by incubation in 0.8 per cent of streptomycin solution similar to the method used in *Group A*. Subcultures of this material yielded numerous colonies after three weeks of incubation, and this culture proved to be resistant to 1 γ of streptomycin per ml.

Group G consisted of 10 rabbits which served as controls. Seven of these were reinfected with 5-mg. doses of untreated strain K-3, and 3 rabbits were reinfected with 5-mg. doses of untreated strain A-1. Subcultures of those materials yielded numerous colonies after two weeks of incubation.

Group H consisted of 6 rabbits which also served as controls. These were given a primary intratracheal inoculation of 5-mg. suspensions of strain K-3 and A-1, some of which had been exposed to PAS, and some treated with distilled water. This was done to obtain a representative control.

The animals were followed by periodic roentgenographic examinations. The individual rabbits were sacrificed at times varying from four days to two months after intratracheal inoculation. The tissues were prepared for microscopic study.

Results

The animals reinfected by living bacilli (*Group G*) developed an extensive, unilateral, evenly concentrated shadow on the chest roentgenogram two days after reinfection. It became slightly enlarged in size, then entirely resolved after five weeks. On the other hand, primarily infected animals (*Group H*) developed no shadow two days after infection, but developed diffuse, unilateral, evenly concentrated or patchy shadows after two or three weeks. Those shadows gradually enlarged. Reinfected animals developed roentgenographic shadows more promptly than primarily infected animals did. In other words, reinfected animals showed hypersensitivities on roentgenograms. Comparison of these hypersensitivities was made between the different groups of animals reinoculated by different inocula, such as PAS- or isoniazid- or streptomycin-exposed bacilli (*Groups A*, *D*, and *F*) heat-killed bacilli (*Group B*), water-treated bacilli (*Groups C* and *E*), and living bacilli (*Group G*). The results are summarized in table 1.

Most of the heat-killed bacilli were considered to be dead, but an early development of shadows resulted, as was expected. It appeared nearly as great as in the case of living bacilli. When reinfection was induced by means of living resistant bacilli A-1 (*Group G*), early developing shadows appeared which showed exactly the same results as the reinfection by living bacilli of strain K-3. When reinfection was made by means of water-treated strain K-3, enough hypersensitivities could be produced though it decreased somewhat. On the other hand, in the case of PAS- or isoniazid-treated bacilli or water-treated resistant bacilli A-1, no strong early developing shadows were recognized, contrary to expectation. Even when shadows appeared they appeared late and were very slight. Furthermore, when rabbits' lungs got primary infection by the above PAS-treated bacilli (*Group H*), after fifteen days it appeared as a very slight shadow which grew gradually larger. But in the case of the streptomycin-treated bacilli K-3, moderately developing shadows were recognized two days after reinfection. Again in the case of primary infection by heat-killed bacilli, no such shadows appeared at all (*Group H*). The rabbits which received primary infection by water-treated bacilli A-1 developed milder shadows than those of the rabbits which received direct infection by living resistant bacilli A-1 (*Group H*).

Pathologic Observation in Rabbits' Lungs

Autopsies performed eight days after reinfection with living tubercle bacilli K-3 revealed tuberculous lobar pneumonia (figure 1). A few tubercle bacilli in this lesion were found on microscopy or culture. The same results were obtained in the reinfected animals with heat-killed bacilli or living resistant bacilli A-1 (figure 2).

Gross and microscopic examinations four days after reinfection with streptomycin- or water-treated tubercle bacilli K-3 revealed tuberculous lobular pneumonia (figures 3, 4). On the other hand, animals reinfected with isoniazid-exposed tubercle bacilli K-3 or water-treated resistant tubercle bacilli A-1 showed a few acinus-sized tuberculous infiltrations eight days after reinfection (figures 5, 6). In other words, the display of a very slight degree of hypersensitivity should

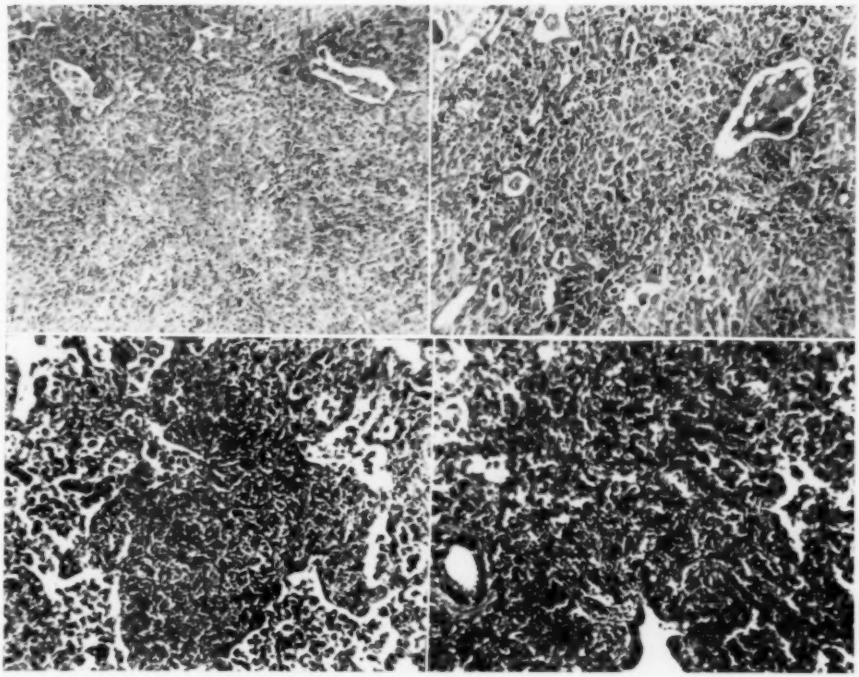


FIG. 1 (Upper left). Tuberculous lobar pneumonia eight days after reinfection with living bacilli K-3. (Hematoxylin and eosin stain, ×150)

FIG. 2 (Upper right). Tuberculous lobar pneumonia eight days after reinfection with living resistant tubercle bacilli A 1. (Hematoxylin and eosin stain, ×150)

FIG. 3 (Lower left). Tuberculous lobular pneumonia four days after reinfection with streptomycin-treated tubercle bacilli K-3. (Hematoxylin and eosin stain, ×150)

FIG. 4 (Lower right). Tuberculous lobular pneumonia infiltration four days after reinfection with water-treated tubercle bacilli K-3. (Hematoxylin and eosin stain, ×150)

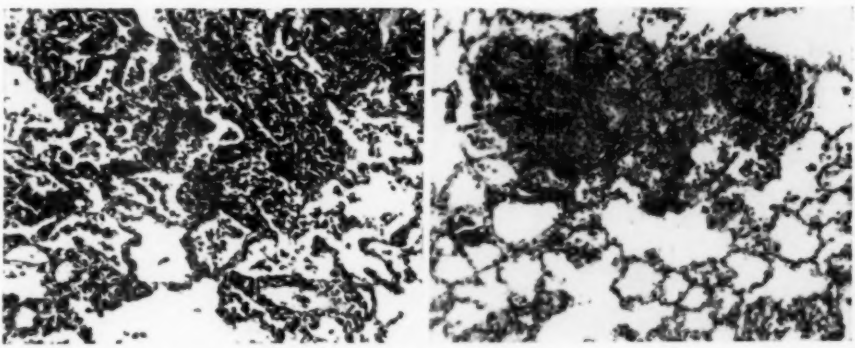


FIG. 5 (Left). Acinus-sized tuberculous infiltration eight days after reinfection with isoniazid-treated tubercle bacilli K-3. (Hematoxylin and eosin stain, ×150)

FIG. 6 (Right). Acinus-sized tuberculous infiltration eight days after reinfection with water-treated resistant tubercle bacilli A-1. (Hematoxylin and eosin stain, ×150)

TABLE 2
KOCH PHENOMENA

Number of Animals	Number of Days														
	1	2	3	4	5	7	8	10	12	13	15	17	20	22	30
Rabbits Reinfected (Challenged) with Isoniazid-treated Bacilli															
1	○ 6 x 7	○ 7 x 7	○ 7 x 7	○ 8 x 9	○ 9 x 9	○ 7 x 9	× 7 x 8	× 7 x 8	× 6 x 6	× 6 x 6	× 4 x 4	× 3 x 3	× 3 x 3	—	—
2	○ 7 x 8	○ 8 x 9	○ 8 x 9	○ 7 x 8	○ 8 x 9	○ 8 x 9	× 8 x 9	× 6 x 7	× 6 x 7	× 6 x 7	× 5 x 6	× 3 x 3	□ 3 x 3	—	—
3	○ 3 x 3	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 7	○ 6 x 6	× 6 x 6	× 6 x 6	× 6 x 6	× 6 x 6	× 5 x 6	× 6 x 6	× 6 x 6	× 4 x 4	× 2 x 2
4	○ 3 x 3	○ 7 x 7	○ 6 x 6	○ 5 x 6	○ 5 x 5	○ 4 x 6	× 5 x 6	× 4 x 6	× 3 x 5	× 4 x 5	× 4 x 5	× 3 x 4	× 3 x 3	—	—
Control Animals. Rabbits Reinfected (Challenged) with Water-treated Bacilli															
5	▲ 4 x 4	▲ 6 x 6	▲ 8 x 8	▲ 9 x 12	● 9 x 11	● 10 x 11	● 9 x 11	□ 9 x 13	□ 8 x 11	□ 9 x 12	□ 9 x 12	□ 7 x 7	□ 6 x 6	□ 4 x 4	× 2 x 2
6	○ 8 x 7	○ 8 x 8	○ 10 x 10	○ 9 x 9	○ 10 x 9	○ 11 x 10	○ 10 x 9	○ 9 x 8	○ 9 x 8	● 8 x 11	● 9 x 11	□ 9 x 11	□ 7 x 7	□ 7 x 7	—
7	○ 9 x 10	○ 9 x 10	○ 11 x 12	○ 11 x 12	○ 11 x 11	○ 11 x 12	○ 12 x 13	○ 11 x 11	○ 12 x 12	○ 12 x 13	○ 11 x 12	○ 10 x 12	○ 8 x 8	○ 8 x 8	○ 8 x 9
8	△ 7 x 9	△ 7 x 9	△ 7 x 9	△ 8 x 8	○ 7 x 7	○ 8 x 9	○ 8 x 9	○ 8 x 9	○ 8 x 9	○ 9 x 10	○ 9 x 9	○ 9 x 9	○ 8 x 8	○ 8 x 8	○ 8 x 8
Guinea Pigs Reinfected (Challenged) with Isoniazid-treated Bacilli															
1	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
2	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
3	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
4	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
Control Animals. Guinea Pigs Reinfected (Challenged) with Water-treated Bacilli															
5	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
7	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6
8	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6	○ 6 x 6

	7 x 9	7 x 9	7 x 9	8 x 8	7 x 7	8 x 9	8 x 9	8 x 9	8 x 9	9 x 10	9 x 9	9 x 9	8 x 8	8 x 8	8 x 8

Guinea Pigs Reinfected (Challenged) with Isoniazid-treated Bacilli

1	○ 5 x 5	○ 5 x 5	○ 6 x 6	○ 6 x 7	○ 8 x 9	○ 9 x 9	○ 8 x 9	○ 8 x 11	○ 8 x 8	○ 9 x 10	○ 8 x 11	○ 8 x 11	○ 7 x 7	○ 4 x 4	
2	○ 12 x 12	○ 12 x 12	▲ 12 x 12	□ 12 x 11	□ 13 x 11	□ 12 x 10	□ 10 x 10	□ 11 x 11	□ 9 x 9	□ 8 x 9	□ 8 x 9	□ 7 x 8	—		
3	○ 4 x 4	○ 4 x 4	○ 5 x 5	○ 4 x 4	○ 4 x 3	○ 4 x 4	○ 4 x 4	● 5 x 5	□ 5 x 5	□ 7 x 7	□ 7 x 7	□ 7 x 7	—		
4	○ 11 x 12	○ 11 x 12	▲ 11 x 12	□ 8 x 12	□ 9 x 13	□ 9 x 12	□ 9 x 9	□ 7 x 7	□ 7 x 7	†					

Control Animals. Guinea Pigs Reinfected (Challenged) with Water-treated bacilli

5	○ 5 x 5	○ 5 x 5	○ 13 x 13	○ 13 x 13	○ 13 x 13	○ 15 x 16	○ 17 x 18	○ 20 x 23	○ 15 x 21	○ 18 x 18	○ 13 x 17	○ 14 x 18	○ 12 x 15	○ 11 x 11	
6	○ 5 x 9	○ 11 x 10	○ 13 x 13	○ 12 x 13	○ 13 x 17	○ 13 x 13	○ 20 x 21	○ 18 x 20	○ 17 x 17	○ 17 x 17	○ 13 x 13	○ 12 x 12	□ 12 x 12	□ 6 x 8	
7	○ 14 x 14	○ 14 x 14	○ 14 x 15	○ 11 x 13	○ 13 x 13	○ 10 x 10	● 11 x 13	● 8 x 10	● 9 x 9	□ 8 x 9	□ 7 x 9	□ 7 x 8	□ 7 x 7	× 7 x 7	

Note: ○—induration △—pustule ▲—abscess ●—ulcer □—crust formation ×—scarring 6 x 7 = 6 mm. by 7 mm.

be considered as evidence that antigenicity was decreased and weakened.

Autopsies performed on some of the groups two months after reinfection revealed isolated foci of caseation which were well encapsulated by fibrous tissue or revealed a few scars.

The animals primarily infected by each of the above-mentioned tubercle bacilli, except heat-killed bacilli, showed either caseous lobar pneumonia or lobe-sized exudative lesions at autopsy after two months. The animals primarily infected by heat-killed bacilli revealed only cellular infiltration of acinus size.

The 8 rabbits were subcutaneously infected with 1 mg. of K-strain of *Mycobacterium tuberculosis*. Five weeks after the infection, 4 of these rabbits were reinfected with 0.25 mg. of isoniazid-treated K-strain via the intracutaneous route. As was the control, the other 4 rabbits were reinfected with 0.25 mg. of water-treated K-strain by the same route.

The results were as follows (table 2):

(*1*) The development of the Koch phenomenon in the rabbits challenged with isoniazid-treated K-strain was less intense than that in the control group challenged with water-treated K-strain.
(*2*) Ulcers were not observed in the rabbits challenged with isoniazid-treated K-strain, but in the control group.
(*3*) The skin lesions healed and scarring developed more rapidly in the group reinfected with isoniazid-treated K-strain than in the control group.

Twenty guinea pigs were subcutaneously infected with 0.05 mg. of K-strain, however. Thirteen of these animals died chiefly from generalized tuberculous infection within five weeks, i.e., before the reinfection.

All of the surviving 7 guinea pigs developed tuberculin reactions. Four of these animals were reinfected with 0.25 mg. of isoniazid-treated K-strain via the subcutaneous route, and the remaining 3 animals were reinfected with 0.25 mg. of water-treated K-strain by the same route.

The results were as follows (table 2):

(*1*) The development of the Koch phenomenon in the guinea pigs challenged with isoniazid-treated K-strain was less intense than that in the control group challenged with the water-treated K-strain.
(*2*) The skin lesion healed more rapidly in the group reinfected with isoniazid-treated K-strain than in the control group.
(*3*) The Koch phenomenon evoked by the isoniazid-treated K-strain was more intense in the guinea pigs than in the rabbits. This might be ascribed to a species difference in terms of sensitivity to *Mycobacterium tuberculosis*.

Discussion

Koch Phenomenon

It was found that typical lobar tuberculous pneumonia was produced by the above-mentioned living tubercle bacilli strain K-3. The rabbits that received primary infection by water-treated resistant bacilli A-1 and PAS-exposed tubercle bacilli K-3 suffered from milder lesions than those that were infected with living bacilli. The living bacilli when cultured showed numerous colonies, but PAS- or isoniazid-exposed tubercle bacilli and water-treated resistant bacilli showed few or no colonies. Accordingly, the fact that infection by bacilli which had been robbed of growth ability was relatively mild reflected a weakened pathogenicity of such bacilli.

Irrespective of whether the tubercle bacilli were living or dead, the rabbits demonstrated hypersensitive reactions as long as the bacilli retained antigenicity. This was shown by reinfection challenges with living bacilli and with heat-killed bacilli. However, in the case of PAS- or isoniazid-exposed bacilli or water-treated resistant bacilli A-1, rabbits did not show a strong hypersensitive reaction, contrary to expectation. This suggests that some of these bacilli did not act as antigens. Both kinds of bacilli when cultured showed only a few colonies. This means that some of them remained as living bacilli although their growth was hampered.

But when rabbits were reinfected by streptomycin- or water-treated tubercle bacilli K-3 whose growth ability had not been weakened by streptomycin or water, a hypersensitive reaction appeared.

It could be accepted that, if growth ability of living bacilli was weakened by contact with PAS or isoniazid, the ability of the bacilli to serve as antigen was also weakened. When resistant bacilli were robbed of their growth ability by exposing them to water, the result was the same. Accordingly, it is considered that a relationship exists between existence of growth ability and ability to serve as antigen. Consequently, when tubercle bacilli resistant to antituberculous drugs, for example streptomycin, were employed in an experiment (*Group F*), the tubercle bacilli retained antigenicity. When

streptomycin was first used, it was feared that a generalized tuberculin reaction due to the products of the drug-killed bacilli might develop similar to the Herxheimer reaction seen in early syphilis. In actuality, there has been a long period of clinical experience since the discovery of streptomycin; yet no report of such a serious case has been recorded. This fact might be explained by the present experiments. There are many reports concerning tubercle bacilli that suffered a diminished ability in the foci of patients treated by antituberculous agents. But these weakened bacilli have weak antigenicity and can not cause a strong allergic reaction even though they might spread intrabronchially.

Summary

When rabbits are reinfected by *Mycobacterium tuberculosis*, whose growth ability has been weakened by PAS or isoniazid, and by resistant bacilli, whose growth ability has been weakened just by keeping them in water, a decrease in the tuberculin reaction appears. By contrast, in the case of a tuberculous infection treated by water or streptomycin, the hypersensitive reaction is preserved to a weaker degree than in an untreated infection.

Primary infection by any of these bacilli gives a milder infection than primary infection by living bacilli, thus proving that there is a decrease in pathogenicity. However, although the decrease of growth ability and the decrease of antigenicity are parallel, antigenicity is preserved in the case of heat-killed bacilli.

It is assumed that the above fact can easily explain the nonexistence of generalized tuberculin reactions, such as the Jarisch Herxheimer reaction in syphilis, that might be caused by tuberculin released from destroyed bacilli in a lesion during chemotherapy. Furthermore, it is worth while noting that the typical Koch phenomenon was produced by the resistant bacilli which were used for the above test.

Sumario

Investigación del Influjo de los Productos Químicos Antibacterianos sobre la Reacción Alérgica a la Tuberculosis de Reinfección en los Conejos

Cuando se reinfecta a los conejos con *Mycobacteria tuberculosis*, cuya capacidad proliferante ha sido debilitada por el PAS o la isoniacida, o con bacilos resistentes, cuya capacidad proliferante ha sido debilitada meramente por la sumersión en agua, aparece una disminución de la reacción hipersensible. En cambio, en lo tocante a la tuberculosis micobacteriana tratada con agua o estreptomicina, se conserva la reacción hipersensible en forma más débil que en la tuberculosis micobacteriana viviente.

La infección primaria por cualquiera de esos bacilos produce lesiones más leves que los bacilos vivos, demostrando así que existe una disminución de la patogenicidad. No obstante, aunque son paralelas la disminución de la capacidad proliferante y la de la antigenicidad, se retiene la antigenicidad cuando se trata de bacilos matados al calor.

Se supone que el hecho anterior puede explicar fácilmente la inexistencia de reacciones generalizadas a la tuberculina, tales como la reacción de Jarisch-Herxheimer en la sífilis, provocadas por la tuberculina liberada por los bacilos destruídos en la lesión bajo la acción de la quimioterapia. Además, vale la pena señalar que fué producido el típico fenómeno de Koch por los bacilos resistentes usados en la prueba descrita.

Resume

Enquête sur l'influence des substances chimiques antibactériennes sur la réaction allergique à la réinfection tuberculeuse chez le lapin

La réinfection du lapin avec la *Mycobactérium tuberculosis*, dont la capacité de croissance a été réduite par le PAS ou l'isoniazide, et par des bacilles résistants, dont la capacité de croissance a été réduite par simple immersion dans l'eau, est suivie d'une diminution de la réaction allergique. D'autre part, dans le cas de la tuberculose due au *Mycobactérium* traité par l'eau ou par la streptomycine, la réaction d'hypersensibilité est conservée à un degré plus faible que dans le cas où il s'agit de tuberculose par inoculation de bacilles vivants.

La primo-infection produite par les bacilles traités donne des lésions moins importantes que la primo-infection causée par des bacilles vivants, ce qui démontre une réduction de la pathogénicité. Toutefois, bien que la réduction de la capacité de croissance et la réduction d'antigénicité soient comparables, l'activité antigénique est conservée lorsque les bacilles sont tués par la chaleur.

On considère que ces faits expliquent facilement l'absence de réactions généralisées à la tuberculine, comme par exemple dans la réaction de Jarisch Herxheimer de la syphilis, quand il s'agit de la tuberculine excrétée par les bacilles détruits provenant de lésions soumises à la chimiothérapie. De plus, il y a lieu de remarquer que le phénomène typique de Koch était produit par

les bacilles résistants qui furent utilisés pour le test précité.

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THE INFLUENCE OF 3,3′,5-TRIIODO-L-THYRONINE ON THE SURVIVAL TIME OF MICE WITH TUBERCULOSIS AND PNEUMOCOCCOSIS[1,2]

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INTRODUCTION

The possible role of the thyroid gland in regulating resistance has been the subject of numerous investigations which, in some instances, have yielded conflicting results depending perhaps on the particular host-parasite model employed. No generalizations can be made at present regarding the relationship of thyroid metabolism to natural resistance.

Izzo and Cicardo (1) investigated the effect of the injection of thyroxine on the course of tuberculosis in guinea pigs. Guinea pigs that were made hyperthyroid and then infected with tubercle bacilli had a longer survival time than did either normal control animals or guinea pigs that had been thyroidectomized. Thyroidectomized animals had an increased susceptibility when compared with control animals. Dubos (2) and Smith and Dubos (3) have reported that the oral administration of thyroid extract to mice decreased their resistance to infection with tubercle bacilli or Staphylococci. Lurie and Ninos (4) noted an increase in resistance of rabbits to tuberculosis when triiodothyronine was administered to the animals in dosages sufficient to increase their basal metabolic rate by 40 per cent.

The effect of 3,3′,5-triiodo-L-thyronine on the resistance of mice to pneumococcosis and tuberculosis, as measured by their survival times, is the subject of this paper.

MATERIALS AND METHODS

Male mice of the CF_1 strain were used and were fed Purina Dog Chow ad libitum. Dosages of 3,3′,5-triodo-L-thyronine (Merck), hereinafter called triiodothyronine, ranged from 3 to 24 γ per 100 ml. of water. The triiodothyronine was given to the animals in their drinking water. Stock solutions of triiodothyronine were prepared every ten days, and from these solutions the various dosages to be given the mice were made. The animals were placed on the drug regimens for periods of two to sixteen days prior to initiation of the infections and were given triiodothyronine at preinfection dosages throughout the course of the experiments. Preinfection basal metabolic rates were determined by employing the closed method as described by Gemmill (5). Survival time data were submitted to the rapid graphic statistical method of Litchfield (6). The 50 per cent survival time was obtained from a plot of cumulative per cent dead versus log time.

The Ravenel strain of *Mycobacterium tuberculosis* var. *bovis* was employed. The organisms were obtained from a seven- to ten-day culture in the glycerol-asparagin broth described by Youmans (7). The organisms were ground with a sterile mortar and pestle and suspended in a solution of Dubos medium containing Tween® 80 (8), diluted 1:10 with saline. This suspension was centrifuged lightly to remove cell aggregates, and the supernatant suspension was adjusted to a reading of 160–165 nephelos units in a Coleman Model 9 nephelometer. Test and control groups of mice consisted of 12 animals from the same lot. Each mouse received 0.5 ml. of this suspension intravenously. The animals that comprised the control groups had not received triiodothyronine.

Standardized suspensions of Type I *Diplococcus pneumoniae* were obtained from an eighteen-hour trypticase soy broth culture diluted for use in trypticase soy broth. The infecting dose (intraperitoneal) consisted of 0.5 ml. of the maximal dilution of an eighteen-hour culture which produced an LD_{100} at thirty hours in normal mice. Test and control groups contained 12 mice from the same lot, and the control animals had not received triiodothyronine.

Peripheral leukocyte counts and differential smears were made on mice undergoing the pneumococcal infection immediately preceding infection and at intervals of six, twelve, and twenty-four hours following infection. Blood was obtained from the incised tails of the mice, and the data from 4 animals in each group were averaged.

RESULTS

Basal metabolic rates: The basal metabolic rates of mice receiving triiodothyronine were

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[2] This study was aided in part by research grants E-1238-C from the Institute of Allergy and Infectious Diseases and A-649 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland.

determined and compared with the rate of normal animals (table 1). The percentage increase ranged from no detectable increase at the 3 γ dosage to an increase of 140 per cent at the 24 γ dosage. The mice remained healthy and could be maintained on these various dosages of triiodothyronine for extended periods of time at room temperature (22 to 25°C.).

Tuberculous infection: A series of five experiments with tuberculous infections was conducted. The results of a typical experiment are presented in table 2 and show that both the 12 and 24 γ dosages produced a decrease in resistance of the mice to the infection, as evidenced by a statistically significant shortening of the survival times when compared with survival times of the control animals. The 3 and 6 γ dosages did not produce a significant difference in the per cent survival time.

A study concerning the effect of reduced preinfection treatment intervals showed that the administration of triiodothyronine for periods of two, four, and eight days at dosages of 3, 6, 12, and 24 γ gave results which were similar to those obtained with a treatment period of sixteen days.

The lungs of tuberculous mice that had received triiodothyronine could not be differentiated from the lungs of control animals when they were examined grossly during the terminal stages of the disease.

Pneumococcic infection: Four experiments were conducted, utilizing pneumococci as the infectious organism. The survival time data for a typical experiment are presented in table 3 and show that the 3 γ dosage did not produce a significant difference in the survival times of the mice. However, the 6, 12, and 24 γ dosages did produce a significant shortening of the survival time. When the mice were treated with triiodothyronine for a period of four days prior to infection with pneumococci, only the 24 γ dosages of the drug produced a significant reduction of the survival times (table 4).

A study of circulating leukocytes in mice with a pneumococcic infection (figures 1 and 2) has shown that the mice receiving triiodothyronine

TABLE 1

PERCENTAGE INCREASE IN BASAL METABOLIC RATE OF MICE RECEIVING TRIIODOTHYRONINE*

Triiodothyronine†	Increment Increase‡
γ	*per cent*
3	0
6	57
12	117
24	140

* Control mice gave values of 8 to 15 cal. per sq. m. per hr.

† Animals treated 8 days before test. Micrograms of triiodothyronine per 100 ml. of drinking water.

‡ The percentage values represent the average of the range of values in each group of 6 mice.

TABLE 2

PER CENT SURVIVAL TIME (ET_{50}) OF MICE INFECTED WITH M. TUBERCULOSIS (RAVENEL)

Group*	ET_{50} in Days	95% Confidence Limits	Significance†
3γ triiodothyronine	28.5	25.7–31.6	NS
6γ triiodothyronine	28.0	26.7–29.4	NS
12γ triiodothyronine	22.5	21.6–23.4	S
24γ triiodothyronine	21.0	20.4–21.6	S
Control	33.0	29.2–38.6	

* Treated 16 days before infection; micrograms of triiodothyronine per 100 ml. of drinking water.

† NS–not significant; S–significant.

TABLE 3

PER CENT SURVIVAL TIME (ET_{50}) OF MICE INFECTED WITH D. PNEUMONIAE

Group*	ET_{50} in Hours	95% Confidence Limits	Significance†
3γ triiodothyronine	25.5	24.7–26.2	NS
6γ triiodothyronine	24.0	23.3–24.8	S
12γ triiodothyronine	22.5	21.6–23.4	S
24γ triiodothyronine	20.5	19.9–21.1	S
Control	28.0	26.6–29.4	

* Treated 8 days before infection; micrograms of triiodothyronine per 100 ml. of drinking water.

† NS–not significant; S–significant.

TABLE 4

PER CENT SURVIVAL TIME (ET_{50}) OF MICE INFECTED WITH D. PNEUMONIAE

Group*	ET_{50} in Hours	95% Confidence Limits	Significance†
3γ triiodothyronine	25.0	22.5–27.7	NS
6γ triiodothyronine	24.0	22.9–25.2	NS
12γ triiodothyronine	21.0	19.3–22.9	NS
24γ triiodothyronine	16.5	14.9–18.3	S
Control	23.5	22.6–24.9	

* Treated 4 days before infection; micrograms of triiodothyronine per 100 ml. of drinking water.

† NS–not significant; S–significant.

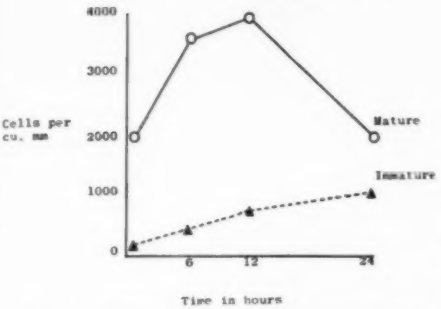

FIG. 1. Polymorphonuclear response of mice infected with *D. pneumoniae*. Normal control group. Animals infected at time 0.

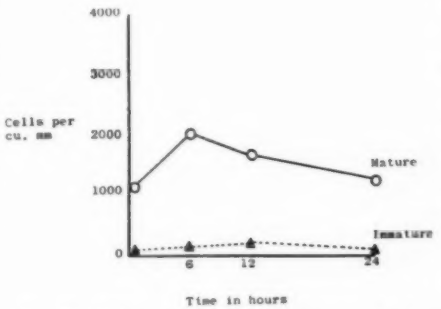

FIG. 2. Polymorphonuclear response of mice infected with *D. pneumoniae*. Mice had received 12 γ of triiodothyronine for eight days before infection. Animals infected at time 0.

had a reduced number of cells of the polymorphonuclear series. The control mice demonstrated an increase in circulating mature polymorphonuclear cells for twelve hours following infection and then the number of these cells decreased until the death of the animals. The number of immature polymorphonuclear cells increased throughout the course of the infection. Mice that received triiodothyronine had fewer circulating mature polymorphonuclear cells before infection than did the control animals. In addition, these mice demonstrated an increase in mature polymorphonuclear cells only during the first six hours of the infection and then the number decreased until the death of the animals. The population of immature polymorphonuclear cells increased only slightly during the course of the infection and decreased after the midpoint of the experiment had been reached.

DISCUSSION

The results of these experiments demonstrate that mice that were made hyperthyroid by the administration of triiodothyronine and then infected with pneumococci or tubercle bacilli exhibited a shortening of the survival time. The 12 and 24 γ dosages of triiodothyronine produced a shortening of the survival times when mice were infected with tubercle bacilli. This pattern did not exhibit a detectable change when the preinfection treatment period was varied over the range of two to sixteen days. With the pneumococcal infection, however, the level of triiodothyronine that caused a shortened survival time varied with the preinfection treatment period employed. When mice were treated for eight days prior to infection, the 6, 12, and 24 γ concentrations produced this effect. When the treatment period was shortened to four days only, the 24 γ dosages of drug caused the survival time of the mice to be significantly reduced. This changing pattern might also have occurred in the case of the tuberculous infections. However, the long-term nature of the tuberculosis experiments could have masked the effect of a shortened pretreatment interval.

The reduction in the number of circulating polymorphonuclear cells in mice that received triiodothyronine could be responsible for the suppressed resistance to pneumococcosis. Once pneumococci are ingested by a phagocytic cell, they are usually destroyed even in the absence of specific antibody (9). If the number of circulating polymorphonuclear cells were subnormal and the animal failed to respond in the usual manner to an infection with the production of additional polymorphonuclear cells, the animal would have a cellular handicap in combating an increasing population of infectious microorganisms.

The relationship between the reduced number of neutrophils and the decreased resistance to tuberculosis of the treated mice is not clear. The role of neutrophils in tuberculosis is generally regarded as a minor one as compared with the important role of the monocyte. Litwack (10) has shown that when rats are treated with thyroid hormone the tissues have a decreased lysozyme activity. Since lysozyme has been demonstrated to inhibit tubercle bacilli *in vitro* (11), this would suggest one possible mechanism for depressing natural resistance to tuberculosis.

The results obtained in this investigation on survival times parallel those of Dubos (2) and Smith and Dubos (3) who produced hyperthyroidism in mice with thyroid extract. On the other hand, the data presented in this paper are not in general agreement with those of Lurie and Ninos (4), who used rabbits and triiodothyronine; or with those of Izzo and Cicardo (1), who used thyroxine and guinea pigs.

The mechanisms by which thyroid hormones influence an animal's resistance to an infection are undoubtedly very complex. This point is exemplified in the variations in results obtained by other investigators with different host-parasite models. It seems probable that triiodothyronine is involved in multiple pathways associated with the physiology of resistance, and different species of animals may respond in qualitatively different patterns. Experimentally this is complicated also by the general observation that animals which have received an exogenous source of this substance demonstrate, over a period of time, a reduction in the size of the thyroid gland; and the animal becomes dependent upon this exogenous source for the greater part of his requirements. The question regarding the effect of triiodothyronine on the resistance of humans to infectious diseases remains unanswered.

Summary

The administration of 3,3′,5-triiodo-L-thyronine to mice in their drinking water caused an increase in the basal metabolic rates of the animals.

Mice that were given triiodothyronine and then infected with tubercle bacilli had a significantly shorter survival time than did normal animals undergoing the same infection.

When mice were treated with triiodothyronine and then infected with pneumococci, it was shown that the mice had a significantly shorter survival time than did normal control animals.

Mice that had been treated with triiodothyronine had a decreased number of circulating polymorphonuclear cells and were not able to respond to a pneumococcal infection with a sustained increase in immature polymorphonuclear cells as did normal control animals.

Sumario

El Influjo de la 3,3′,5-Triyodo-L-Tironina sobre el Tiempo de Sobrevivencia de los Ratones con Tuberculosis y Neumococosis

La administración de 3,3′,5-triyodo-L-tironina a los ratones en el agua de bebida ocasionó un aumento de las tasas del metabolismo basal de los animales.

Los ratones que recibieron triyodotironina y fueron luego infectados con bacilos tuberculosos mostraron un tiempo de sobrevivencia decididamente menor que el de los animales normales que experimentaron la misma infección.

Cuando se trató a los ratones con triyodotironina y se les infectó luego con neumococos, se observó que los animales mostraban un tiempo de sobrevivencia decididamente menor que el de los animales normales que servían de testigos.

Los ratones que habían sido tratados con triyodotironina tenían un número menor de glóbulos polimorfonucleares en la circulación, y no eran capaces de responder a una infección neumocócica con un aumento sostenido de polimorfonucleares inmaduros como hacían los testigos normales.

Resume

L'influence de 3,3′,5-Triiodo-L-Thyronine sur le temps de survie des souris atteintes de tuberculose et de pneumococcie

La 3,3′,5-triiodo-L-thyronine administrée aux souris dans l'eau de boisson augmentait le taux du métabolisme basal de l'animal.

Après avoir reçu la triiodothyronine les souris qui étaient inoculées avec le BK avaient un temps de survie significativement plus court que les animaux normaux ayant subi la même inoculation.

Quand les souris traitées par la triiodothyronine étaient inoculées ensuite avec le pneumocoque, on observait que le taux de survie des souris était significativement plus court que celui des animaux-témoins normaux.

Chez les souris ayant été traitées par la triiodothyronine, le nombre des polymorphonucléaires circulants était réduit, et l'animal était incapable de répondre à l'infection pneumococcique par un accroissement soutenu des cellules polymorphonucléaires jeunes, comme c'était le cas chez les animaux-témoins normaux.

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A CRITICAL ANALYSIS OF THE ACCURACY OF A CHEMICAL AND BIOLOGIC METHOD OF DETERMINING SERUM-FREE ISONIAZID[1]

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Introduction

The use of high doses of isoniazid in the treatment of tuberculosis has recently stimulated increasing interest, and a number of investigations of the subject are in progress (1). Investigators searching for a possible enhanced treatment result with high doses of isoniazid will also wish to determine whether or not adequate concentrations of the drug are detectable in the serum. As will be shown later in this paper, a considerable number of patients acetylate isoniazid at a rapid rate and leave a very small proportion of a given dose of the drug in the serum as "free," biologically active isoniazid. In order to determine accurately the clinical effect of elevated doses of isoniazid, it is necessary to correlate the dose of isonazid with the serum concentration of biologically effective isoniazid. Therefore, in all investigations into the effect of high doses of the drug, the accurate determination of free, unacetylated isoniazid seems to be of paramount importance. This present report specifically concerns a critical evaluation of the accuracy of a biologic and a chemical method of measuring free, unacetylated serum isoniazid concentrations.

Materials and Methods

In the present report, 1,376 serum determinations of free isoniazid and 201 serum determinations of total isoniazid were made by the chemical method. Determinations of free-isoniazid serum concentrations by the biologic assay method were made on 237 specimens of serum. All patients were given either 10, 16, or 18 mg. of isoniazid per kg. per day in three equally divided doses. In addition, each patient received 5.57 gm. of sodium para-aminosalicylic acid (equivalent to 4 gm. of PAS-acid) three times daily. On the morning of the test, one-third of the total daily dose of isoniazid and PAS was administered at 6:00 a.m. Between 7:30 and 8:00 a.m., the patient was permitted a light breakfast. Exactly six hours later, at 12:00 noon, blood was drawn and placed in a sterile test tube. Therefore, all isoniazid concentrations reported in tables 1, 1A, 2, and 4 through 8 reflect the serum concentrations of drug present six hours after the administration of one-third of the total daily dose of isoniazid and PAS. The control determinations reported in table 3 represent serum taken from patients who had not received isoniazid or PAS for a minimum of seventy-two hours prior to the test.

Free-isoniazid concentrations reported by the chemical method of analysis were reported in exact numbers. Since the biologic assay method is a serial dilution technique, results of free isoniazid by this method were reported within a range of two tubes as follows: less than 0.15, 0.15–0.3, 0.3–0.6, 0.6–1.2, 1.2–2.4, 2.4–4.8, and 4.8–9.6 γ per ml. of serum.

The standard deviation for each method of analysis (tables 2 through 8) was determined by extracting the square root of the average of the sum of the squares of each of the test deviations. As will be observed in the tables, the standard deviation is a measurement of precision. It may be predicted that 95 per cent of test results will fall within a range equal to twice the standard deviation.

In table 2, the deviations that are squared are the deviations of the averages of the free-isoniazid range concentrations reported in the initial and duplicate tests. (Example: Initial test 0.3–0.6, average 0.45; duplicate test 0.6–1.2, average 0.9; deviation equals 0.9 minus 0.45, or 0.45.) In table 3, the squared deviations are the actual deviations from zero. In tables 4, 5, and 7, the deviations squared are the actual mathematical differences between the initial and duplicate tests. In tables 6 and 8, the squared deviations represent the actual plus or minus deviations of the chemical determinations from either extreme of the reported bioassay range concentrations.

All duplicate determinations were performed by the double blind method. For example: 25 to 30 ml. of venous blood were obtained from a patient; half of this blood was placed into each of two sterile test tubes, and both tubes plus laboratory request slips were stamped with the name, ward, and hospital number of different patients. In this way, only the physician recording the data had knowledge of the origin of the serum tested.

The method used for the quantitative chemical determination of free isoniazid in serum was basically that of Kelley and Poet (2), as modified and expanded by Maher and associates (3). The latter procedure permitted the determination of both free and acetyl forms of the drug as well as

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the determination of PAS. Although only the concentrations of free isoniazid are of interest in this study, both free and acetyl-isoniazid concentrations were determined in the initial stages of the survey.

In the method of Maher, an aliquot of serum is simultaneously deproteinized with ammonium sulfate and extracted with an organic solvent by horizontal shaking and followed by centrifugation. The organic solvent is a mixture of isoamyl alcohol and ethylene dichloride. Isoniazid (free and acetylated) is extracted from the solvent with dilute hydrochloric acid; PAS is similarly extracted with dilute sodium hydroxide. The determination of free isoniazid is accomplished colorimetrically by treating the acid layer with an alcohol solution of vanillin (3-methyl-4-hydroxybenzaldehyde). Total isoniazid is determined colorimetrically on the same acid-vanillin solution following acid-heat hydrolysis for one hour with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde). If PAS need be determined, the alkali layer is treated similarly with Ehrlich's reagent. Throughout the analysis a blank of drug-free serum and a series of standards are treated similarly. The standards are prepared by adding known amounts of isoniazid, acetyl-isoniazid, and PAS to the same drug-free serum as is used for the blank. A calibration curve is prepared from these standards by plotting optical density vs. concentration of the constituents in micrograms per milliliter of serum.

Minor modifications were made in Maher's method, and these are essentially as follows: (*1*) Instead of the more expensive 50-milliliter glass-stoppered centrifuge tubes, similar tubes equipped with polyethylene snapcaps were substituted.[2] Further advantages were found to be the nonbreakable and nonfreezing qualities of the plastic cap. (*2*) A serum blank, using the same drug-free serum as that used in preparation of the serum standards, was carried through the procedure. Optical densities of this serum blank, the standards, and the serum specimens were read against pure 0.1 normal hydrochloric acid after prescribed treatment with vanillin. The latter acid was not treated with vanillin and was used to set the Beckman DU spectrophotometer at zero density. The optical density of the drug-free serum blank was then subtracted from each of the optical densities of serum standards and serum specimens.

In the early stages of the survey, a control serum was run on each patient. This specimen was drawn seventy-two hours after isoniazid and PAS had been discontinued. Its purpose was to take into account the presence of nonspecific materials in the serum of untreated patients which react, as free isoniazid, with the reagents used in the procedure.

In the microbiologic assay for isoniazid in serum (4, 5), twofold serial dilutions of serum beginning with a 1:5 dilution were made for ten tubes in liquid RDM-3 medium (5). All tubes were inoculated with 0.05 ml. of a seven-day culture of isoniazid-susceptible tubercle bacilli, strain H37Rv, grown in RDM-2 medium (5). Standard isoniazid control dilutions were made to contain a final concentration of: 0.1, 0.08, 0.04, 0.02, 0.01, 0.005, and 0.1 γ of isoniazid per ml. of medium. The same test suspension of *M. tuberculosis* was added as in the serum dilutions.

Aseptic techniques must be used throughout all phases of the procedure. The tubes with serum dilutions were read in twelve days for macroscopic evidence of growth and compared with the standard isoniazid series. The concentration of isoniazid in the tubes containing serial dilutions of serum can be determined by dividing the concentration of isoniazid which inhibits growth in the control series by the dilution fraction in the test series. For example, if a concentration of 0.03 γ of isoniazid per ml. inhibited the H37Rv strain in the control, and there was no growth in the 1:20 dilution with growth in the 1:40 dilution of serum, then the patient's serum isoniazid concentration would be calculated to be 0.6 to 1.2 γ per ml.

Results

Specimens of the serum of 201 patients taking high doses of isoniazid were subjected to chemical analyses, as shown in table 1. Free and total isoniazid concentrations were determined six hours after one-third of the total daily dose of iosniazid and PAS was administered. The total percentage of conjugated forms of isoniazid are reported as acetyl-isoniazid. Therefore, the percentages of acetyl-isoniazid recorded in table 1 were calculated with relation to the total amount of all forms of isoniazid present in the serum six hours after the drug was administered orally. For example, the 39 patients listed in table 1 who, after six hours, had conjugated more than 90 per cent of the isoniazid available in their serum had less than 10 per cent of their serum

TABLE 1

Rates of Acetylation of Isoniazid
201 Patients

Per Cent Acetyl-Isoniazid	Very Rapid 90–100%	Rapid 75–90%	Moderate 50–75%	Slow 25–50%	Very Slow 0–25%
Number of patients	39	39	36	61	27
Percentage of total 201 patients	19.4	18.9	17.9	30.4	13.4

38.3%
201 patients

[2] These items were obtained from Kimble Glass, Stock No. 45205 (Exax tube) and No. PS-45 (cap).

TABLE 1A

FREE ISONIAZID SERUM CONCENTRATIONS IN 5 SELECTED PATIENTS

(Reported in γ per Ml. of Serum)

	Hours After ½ Total Daily Dose of Isoniazid and PAS		
	2 Hours	5 Hours	6 Hours
Very rapid inactivator	1.2	0.3	0.15
Rapid inactivator	2.4	0.6	0.3
Moderate inactivator	2.4	0.6	0.6
Slow inactivator	4.8	2.4	2.4
Very slow inactivator	9.6	4.8	4.8

isoniazid in the form of free, biologically active isoniazid. The 201 patients listed in table 1 were divided into five groups according to the percentage of total drug in the serum which had been chemically inactivated after six hours. Very rapid acetylation of isoniazid occurred in 19.4 per cent of cases. Rapid acetylation, with more than 75 per cent conjugation of free isoniazid occurred in 38.3 per cent of cases. From this table it may be determined that 56.2 per cent of 201 patients had inactivated more than one-half of the isoniazid present in their serum six hours after oral administration of the drug.

Serial determinations for isoniazid in serum after a single oral dose of 5 mg. of isoniazid per kg. plus 4 gm. of PAS have demonstrated the following: the concentration of isoniazid in the serum rises rapidly and reaches a peak in approximately one and a half to two hours; there is then a gradual decline in the serum isoniazid concentration over the next four hours; six hours after an oral dose of isoniazid there is a much more gradual decline in serum isoniazid concentrations. Determinations of serum isoniazid concentrations are obtained six hours after an oral dose because serum concentrations of the drug are more constant at this time. This situation is illustrated in table 1A, which also illustrates the pattern found in rapid, medium, and slow "acetylators." In this table, serum concentrations of free isoniazid in 5 selected patients were determined by the biologic assay method. The lowest concentration of the range as reported by the serial dilution technique is listed, i.e., if the free isoniazid concentration was reported within a range of 2.4 to 4.8 γ per ml., the concentration was reported to be 2.4. All patients received one-third of a total daily dose of 16 mg. of isoniazid per kg. and 12 gm. of PAS.

The results of duplicate free-isoniazid serum concentrations performed by the biologic method on 65 separate serum samples are summarized in table 2. Exact duplication of serum concentrations was made in 52 (80 per cent) of patients. In thirteen specimens of serum, concentrations were not duplicated exactly, but the differences between the initial and duplicate tests were in error only by a difference of one tube in the serial dilution method of the test. The standard deviation for the method was 0.48.

In tables 3 through 6, the analysis of samples of patients' serum by the chemical assay method is set forth. In this phase of the study, all determinations were performed by a research chemist in a research laboratory. The motivation and conditions under which tests were performed were ideal. The serum samples of 182 patients were examined for free isoniazid even though the drug was not being administered at the time (*see* table 3). This phase of the study was initiated to

TABLE 2

65 DUPLICATE FREE-ISONIAZID SERUM CONCENTRATIONS BY THE BIOLOGIC METHOD

Number of Cases	Initial Test	Duplicate Test	Percentage of Total Cases
	γ *per ml.*	γ *per ml.*	
5	0.15–0.3	0.15–0.3	
17	0.3–0.6	0.3–0.6	
5	0.6–1.2	0.6–1.2	80
1	1.2–2.4	1.2–2.4	
15	2.4–4.8	2.4–4.8	
9	4.8–9.6	4.8–9.6	
4	0.15–0.3	0.3–0.6	
2	0.3–0.6	0.6–1.2	
4	0.6–1.2	1.2–2.4	20
1	1.2–2.4	2.4–4.8	
2	2.4–4.8	4.8–9.6	

Standard deviation = 0.48.

TABLE 3

182 CONTROL DETERMINATIONS FOR FREE ISONIAZID BY THE CHEMICAL METHOD

(All Serum Samples from Patients not Taking Isoniazid)

Number of Cases	Concentrations Reported in γ per Ml.	Percentage of Total 182 Cases
101	0	55.6
37	0.1–0.4	20.3
37	0.5–0.9	20.3
7	1.0–1.7	3.8

Standard deviation = 0.41.

TABLE 4

59 DUPLICATE FREE-ISONIAZID DETERMINATIONS BY THE CHEMICAL METHOD

(Initial Test Performed on 3 Ml. of Serum)

(Duplicate Test Performed on 3 Ml. of Serum)

Number of Tests	Inclusive Range of Initial Tests	Actual Deviation of Each Duplicate Test From Initial Test
	γ per ml.	γ per ml.
4	0.0	0.0; 0.0; 0.0; 2.0
3	0.1–0.4	0.2; 0.2; 0.5
8	0.5–0.9	0.1; 0.1; 0.2; 0.2; 0.4; 0.5; 0.8; 0.9
5	1.0–1.4	0.0; 0.2; 0.2; 0.3; 0.8
9	1.5–1.9	0.1; 0.2; 0.3; 0.3; 0.5; 0.5; 0.6; 0.7; 0.8
4	2.0–2.4	0.1; 0.3; 0.5; 0.6
4	2.5–2.9	0.1; 0.3; 0.7; 0.8
5	3.0–3.9	0.0; 0.1; 0.4; 0.7; 1.0
5	4.0–4.9	0.0; 0.1; 0.3; 0.8; 1.0
5	5.0–5.9	0.1; 0.2; 0.3; 0.4; 1.3
5	6.0–6.9	0.0; 0.3; 0.4; 0.6; 1.9
2	7.0–11.4	0.1; 0.1

Standard deviation = 0.5.

TABLE 5

45 DUPLICATE FREE-ISONIAZID DETERMINATIONS BY THE CHEMICAL METHOD

(Initial Test Performed on 3 Ml. of Serum)

(Duplicate Test Performed on 2 Ml. of Serum)

Number of Tests	Inclusive Range of Initial Tests	Actual Deviation of Each Duplicate Test from Initial Test
	γ per ml.	γ per ml.
8	0.0	0.0; 0.0; 0.0; 0.0; 0.0; 0.6; 1.0; 1.2
3	0.1–0.4	0.5; 0.7; 1.7
8	0.5–0.9	0.2; 0.2; 0.5; 0.5; 0.7; 0.8; 0.8; 0.9
6	1.5–1.9	0.1; 0.3; 0.4; 0.6; 0.9; 1.7
3	2.0–2.4	0.5; 0.7; 0.8
3	2.5–2.9	0.3; 0.5; 0.6
4	3.0–3.9	0.4; 1.1; 1.3; 1.9
7	4.0–4.9	0.0; 0.1; 0.4; 0.4; 0.5; 0.5; 1.3
2	5.1–5.5	0.5; 1.0
1	6.8	1.1

Standard deviation = 0.8.

check the basic accuracy of the chemical method and to consider the possible presence of non-specific substances that could be erroneously interpreted as available free isoniazid. As noted, 55.6 per cent of the patients had no detectable substances recorded as free isoniazid. In 96.2 per cent of all serum samples, the maximal amount of "apparent" free isoniazid reported was 0.9 γ per ml. or less. In the serum of only 7 patients (3.8 per cent) were the concentrations reported to be in gross error. The standard deviation for this method was 0.41.

Duplicate free-isoniazid concentrations were determined on the serum specimens of 59 patients, as illustrated in table 4. This phase of the study was performed to evaluate the chemical method of analysis with respect to its accuracy in the duplication of results on the same serum sample. The initial and duplicate tests were each performed on separate 3.0-ml. samples of the same serum. Actual deviations of 0.4 or less occurred in 71.2 per cent, and 95 per cent of deviations were not greater than 1.0 γ per ml.

In table 5, a similar study performed on 45 samples of serum is described. The only difference from the method described in table 4 and that in table 5 is that the duplicate tests in table 5 were performed on 2.0 ml. of serum rather than on 3.0 ml. Only 35.6 per cent of results could be duplicated within a maximal difference of 0.0 to 0.4 γ per ml. The standard deviation was

TABLE 6

COMPARISON OF CHEMICAL AND BIOLOGIC METHODS OF DETERMINING SERUM-FREE ISONIAZID

(Duplicate Assay on 26 Serum Samples)

Initial Test Chemical Method	Duplicate Test Biologic Method	Deviation of Chemical Determination from Reported Biologic Range of Free Isoniazid
γ per ml.	γ per ml.	
0.0	0.6–1.2	0.6
0.0	0.6–1.2	0.6
0.0	1.2–2.4	1.2
0.1	0.6–1.2	0.5
0.2	0.15–0.3	0.5
0.2	0.6–1.2	0.4
0.6	0.6–1.2	0.0
0.6	0.15–0.3	0.3
0.7	1.2–2.4	0.5
1.1	0.6–1.2	0.0
1.1	1.2–2.4	0.1
1.1	1.2–2.4	0.1
1.8	1.2–2.4	0.0
2.0	2.4–4.8	0.4
2.6	1.2–2.4	0.2
2.8	2.4–4.8	0.0
2.9	2.4–4.8	0.0
3.4	2.4–4.8	0.0
3.6	2.4–4.8	0.0
3.8	2.4–4.8	0.0
4.1	2.4–4.8	0.0
4.3	2.4–4.8	0.0
4.4	2.4–4.8	0.0
4.4	4.8–9.6	0.4
4.8	4.8–9.6	0.0
5.1	4.8–9.6	0.0

Standard deviation = 0.36.

0.5 when 3.0 ml. of serum were used for both initial and duplicate tests. When less than 3.0 ml. of serum were used, the standard deviation was 0.8, or 1.6 times as great.

The results of duplicate assay tests for free isoniazid on the same serum specimen is also illustrated in table 6. However, in this study, the initial test was performed by the chemical method and the duplicate test by the biologic method. The deviation of the chemical test from the reported biologic range concentration did not exceed 0.6 γ per ml. in 25 of 26 specimens of serum. The standard deviation for this method was 0.36.

The final phase of the present paper concerns the accuracy of chemical determinations of serum-free isoniazid when not performed by a research chemist under research laboratory conditions. The regular hospital chemist was instructed in the chemical method of analysis described herein. After a trial period for purposes of familiarization, a series of controlled, duplicate analyses was made. This chemist was asked to perform the isoniazid determinations in addition to his duties of performing the multitude of other chemical analyses common to a general hospital. The results are summarized in tables 7 and 8. In table 7, 25 serum specimens were tested by duplicate analysis using the chemical method.

TABLE 7

25 Duplicate Free-Isoniazid Determinations by the Chemical Method

(Initial and Duplicate Tests Performed on 3-Ml. Samples of Same Serum)

Number of Tests	Inclusive Range of Initial Tests	Actual Deviation of Each Duplicate Test from Initial Test
	γ per ml.	γ per ml.
4	0.5–1.0	0.0; 0.2; 1.6; 1.6
7	1.1–1.5	0.1; 0.1; 0.2; 0.3; 0.4; 0.9; 1.0
2	2.0–2.4	0.0; 0.8
2	4.4–4.8	0.1; 1.3
4	5.1–5.5	0.0; 0.3; 0.3; 2.3
4	7.2–8.2	0.1; 0.6; 1.4; 5.9
2	9.4–11.7	1.3; 2.5

Standard deviation = 1.55.

Only 60 per cent of serum samples could be duplicated within a difference of 0.8 γ per ml. or less. In as many as 32 per cent, the actual difference between initial and duplicate tests was as much as 1.1 to 5.9 γ per ml. The standard deviation for this analysis was 1.55.

Free-isoniazid concentrations were obtained in 81 specimens of serum by the chemical method, and a duplicate determination of free isoniazid was performed on each of these 81 samples by the biologic method. As noted in table 8, 43.2 per cent of chemical tests deviated from the bioassay

TABLE 8

Comparison of Chemical and Biologic Determinations of Free Isoniazid

(81 Separate Serum Samples)

Number of Serum Samples Tested	10	24	9	6	18	14
Biologic concentration on each serum sample (γ per ml.)	0.15–0.3	0.3–0.6	0.6–1.2	1.2–2.4	2.4–4.8	4.8–9.6
Chemical concentration on each serum sample (γ per ml.)	0.5	0.6; 0.8	1.0	1.4	3.3; 3.7	4.1
	0.6	0.8; 0.9	1.2	1.5	3.7; 4.4	6.9
	0.9	1.0; 1.0	1.4	2.4	4.8; 4.8	7.1
	1.0	1.0; 1.1	1.4	3.1	5.1; 5.2	7.1
	1.1	1.4; 1.4	1.5	3.4	5.5; 5.5	7.2
	1.3	1.4; 1.5	2.0	3.7	5.9; 6.4	7.3
	1.4	1.5; 1.6	2.0		6.6; 6.8	8.0
	1.6	1.7; 1.7	2.2		7.5; 7.8	8.0
	2.1	1.8; 1.8	2.5		7.7; 8.2	9.4
	3.3	1.9; 2.0				9.7
		2.1; 2.4				10.4
		2.5; 2.7				11.1
						11.4
						11.7

Standard deviation: 1.18.

range by a difference of 0.0 to 0.4, and only 63 per cent were within a maximal difference of 0.0 to 0.9 γ per ml. Thirty-seven per cent grossly differed by as much as 1.0 to 3.4 γ per ml. The standard deviation for the results in table 8 was 1.18.

Discussion

The data in tables 1 and 1*A* indicate the quantitative and qualitative aspects of inactivation of biologically active isoniazid. Although 38.3 per cent of 201 patients acetylated the main portion of their circulating isoniazid (table 1), this certainly does not imply that 38.3 per cent of patients with active tuberculosis will be treatment failures. It may be theorized that those treatment failures which do occur are more likely to be represented in that group of patients who acetylate the drug at a more rapid rate. If a patient is determined to be a rapid inactivator of isoniazid, he may have a better opportunity for recovery with 10 to 18 mg. of isoniazid per kg. per day than with 4 mg. per kg. daily. The clinical experiences of the present writers with high doses of isoniazid plus PAS have tended to lend support to this contention.

The microbiologic assay for isoniazid in serum has been used by the Research and Development Service of Fitzsimons Army Hospital for the past three years. It has been indicated (personal communication) that results by this method of assay for serum-free isoniazid can be duplicated fairly consistently. All errors in duplication are limited to a difference of one tube in the serial dilution technique of the method. This has again been demonstrated by the results of duplicate analyses on the serum samples of 65 patients enumerated in table 2.

The data described in tables 3, 4, and 6 indicate that the chemical method of assay of free isoniazid described herein appears to be a reliable method. Duplicate tests on the same sample of serum produced results that were quite similar to the small percentage of deviations observed in the biologic method. The standard deviation for analyses in which the chemical method was used (tables 3, 4, and 6) varied between 0.36 and 0.5. The standard deviation for duplicate analyses performed by the bioassay method was 0.48. It appears that both the chemical and biologic methods of analysis are dependable and of equal accuracy. The results described in table 5 merely indicate that chemical determinations made on less than 3 ml. of serum are inaccurate and approximately double the error.

The results in tables 7 and 8 indicate that the accuracy of the chemical method deteriorates when not accepted as a research project. The method is basically not a difficult one. However, with the double-extraction technique plus preparation of standards, it may become difficult when compared to other less complicated chemical determinations performed in the average clinical laboratory. The duplication of results in tables 7 and 8 was approximately three times less accurate than results obtained when the procedure was accepted as a research project on a full-time basis.

If table 3 is scrutinized, it can be calculated that "apparent" serum concentrations of free isoniazid were reported between 0.1 and 0.4 γ per ml. in 20.3 per cent; between 0.5 and 0.9 in 20.3 per cent; and between 1.0 and 1.7 in 3.8 per cent. The 20.3 per cent of patients reported to have concentrations in excess of 0.4 γ per ml. would be absorbed in the basic degree of accuracy as calculated for the analysis. The 20.3 per cent of serum samples reported to have "false" free-isoniazid concentrations between 0.5 and 0.9 γ per ml. also would not change the accuracy of the chemical analysis if the following hypothesis were adopted. It has been thought that an adequate concentration of serum-free isoniazid six hours after the administration of one-third of the total daily dose of the drug is probably in the range of 0.6 to 0.8 γ per ml. By adding a maximal error 0.9 to this concentration, it may be assumed that any chemical concentration of 1.5 to 1.7 or more is adequate, regardless of the presence of interfering substances reported as free isoniazid. The maximal deviation of test results in table 3 was 1.7, and this occurred in only 7 (3.8 per cent) of 182 patients. This error would only be of concern clinically when concentrations of free isoniazid of less than 2.5 were reported. In a separate study of 198 chemical determinations in patients receiving high doses of isoniazid, 64.6 per cent of concentrations were less than 2.5 γ per ml. after six hours. Therefore, the determinations in 3.8 per cent of this group, or actually only 2.4 per cent of patients with serum concentrations of less than 2.5, might be in gross error. The accuracy of the chemical method might be made more finite by first determining the amount of nonspecific substances present in the serum and

reportable as free isoniazid by the chemical method of analysis. This amount could then be subtracted from the serum-free isoniazid concentration reported after the patient was administered the drug. The result might more truly reflect the actual amount of free isoniazid in the serum and eliminate the gross error possible in 3.8 per cent of patients. Under these circumstances, the chemical method of assay could be considerably more accurate than the biologic method. It would first be necessary to determine if these nonspecific substances reportable as free isoniazid are constant. If they are not constant, subtracting their result from a determination made three or more days later on the same patient would merely introduce another error.

Summary

Rapid acetylation of isoniazid occurred in 38.3 per cent of 201 patients. It is this group of patients who might be considered potential treatment failures when conventionally lower doses of isoniazid are administered.

The chemical and biologic methods of assay of serum-free isoniazid as described herein are dependable techniques and encompass an acceptable and equal accuracy for clinical use.

The chemical method of determining serum-free isoniazid described herein is an accurate one only when utilized as a research procedure. It cannot be accepted with equal reliability when given to the average clinical laboratory technician charged with many other responsibilities.

Sumario

Análisis Crítico de la Exactitud de un Método Químico y Biológico para Determinar la Isoniacida Libre en el Suero

En 38.3 por ciento de 201 enfermos fué rápida la acetilación de la isoniacida. Los enfermos de este grupo son los que podrían considerarse como fracasos potenciales del tratamiento cuando se les administran dosis convencionalmente más bajas de isoniacida.

Los métodos químicos y biológicos de valoración de la isoniacida libre en el suero en la forma descrita son técnicas fidedignas y dotadas de exactitud aceptable e igual para empleo clínico.

El método químico descrito aquí para determinar la isoniacida libre en el suero es exacto únicamente cuando se utiliza como procedimiento de investigación. No cabe aceptarlo con igual seguridad cuando se confía a la mayoría de los laboratorios clínicos como una tarea más sobrepuesta a otras obligaciones.

Resume

Analyse critique de la précision de la méthode chimique et biologique dans la détermination de l'isoniazide sérique libre

L'acétylation rapide de l'isoniazide s'est produite dans 38.3% des cas parmi un groupe de 201 patients. Ce sont ces cas que l'on peut considérer comme un potentiel d'échec thérapeutique quand on administre les doses usuelles basses d'isoniazide.

Les méthodes de détermination biologiques et chimiques de l'isoniazide sérique libre, telles qu'elles sont décrites dans cet article, constituent des techniques sûres et d'une précision égale et satisfaisante pour l'usage clinique.

La méthode chimique décrite dans l'article et qui permet de déterminer l'isoniazide sérique libre n'offre de précision que lorsqu'elle est utilisée comme procédé de recherche. Elle ne peut être acceptée avec une même sécurité quand elle est exécutée par un laboratoire clinique ordinaire, comme un examen de routine parmi tant d'autres.

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THE TOLERABILITY OF CALCIUM BENZOYL-PAS AND CALCIUM PAS[1]

A Double Blind Study

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Introduction

The drug para-aminosalicylic acid (PAS) has played an important part in the management of tuberculosis ever since 1946, and now enjoys widespread usage as a companion drug for isoniazid or streptomycin. Moreover, it has been reported that PAS may inhibit the rate of acetylation of isoniazid (1). In view of the increasing importance of long-term tuberculosis chemotherapy, however, greater difficulties are being met because of the large doses of PAS required and the subsequent limited gastrointestinal tolerability. For this reason, efforts are being made to improve the tolerability of PAS by using new pharmaceutical forms, different salts (2, 3), or by incorporating buffers (4). An attempt was made in France with the introduction of benzoyl-PAS (B-PAS) (5). The calcium salt of this PAS derivative is practically tasteless and can be administered without coating (6). Weight for weight, it has proved as effective as PAS in clinical activity (7, 8), in delaying the emergence of drug-resistant tubercle bacilli (9), and in influencing isoniazid acetylation (10).

Several writers (9, 11) have repeatedly stated that calcium B-PAS is relatively nontoxic and is preferred by patients because of its acceptable taste. While almost all writers (6–8, 11) have found that B-PAS produces less gastrointestinal disorders, the findings of Phillips and co-workers (9) do not agree; indeed, with the current daily dose of 12 gm., they saw no difference in the tolerability of a calcium B-PAS suspension and a sodium PAS solution. In order to elucidate the discrepancy between the findings of Phillips and co-workers and those of the other writers, the question of the tolerability of calcium B-PAS has been examined in a double blind test.[2]

[1] From the Bernese Sanatorium for Tuberculous Patients, Heiligenschwendi, and the Research Institute of Dr. A. Wander S. A., Berne, Switzerland.

[2] The helpful suggestions of Dr. Samuel Phillips, Chief, Pulmonary Disease Section, Medical Teaching Group Hospital, Veterans Administration, Memphis, Tennessee, in the experimental design, are gratefully acknowledged.

Plan of Investigation

All patients with pulmonary tuberculosis admitted to the Bernese Sanatorium, Heiligenschwendi, between March, 1957, and February, 1958, and suitable for comparative PAS therapy according to their condition and the findings of the drug-resistance tests, were included in the trial. Patients with odd case history numbers were given benzoyl-PAS; those with even-numbered case histories were given PAS. Both drugs were administered as the calcium salt. In Switzerland, severe and moderately advanced cases of pulmonary tuberculosis are first subjected to multiple-drug therapy, including intravenous PAS infusions. There was, therefore, a certain delay in the inclusion of some of the patients in this study. Throughout the trial, the identity of the PAS preparation administered was unknown to the patients, nursing personnel, and physicians, as well as to the investigators who evaluated the results. The need for combined treatment was met by incorporating isoniazid in doses shown by experience to produce side effects very rarely. To increase the difficulty of differentiating between the preparations and to eliminate the factor of taste, both PAS–isoniazid combinations were given in identical cachets.[3] Each cachet contained either 1.25 gm. of calcium B-PAS (equivalent to 1 gm. of acid B-PAS) and 25 mg. of isoniazid, or 1.31 gm. of calcium PAS (equivalent to 1 gm. of acid PAS) and 25 mg. of isoniazid. For a continuous period of twelve weeks, patients weighing up to 50 kg. were given 4 cachets twice daily; those weighing up to 70 kg. received 4 cachets three times daily; and patients weighing more than 70 kg. were given 4 cachets four times daily. Dosage based on body weight appears to be more satisfactory than the administration of standard doses of PAS and B-PAS (7). Thus, underweight patients are not unduly strained, while insufficient dosage is avoided in overweight patients. If either PAS preparation was poorly tolerated, the daily dose was temporarily reduced to 8 gm. If this measure failed to control the disturbance, a change-over was made, if possible, usually within the first few weeks. If the patient proved intolerant to both preparations, PAS therapy was discontinued. Changes in dosage, as well as the incidence of subjectively reported or objectively observed side effects, were recorded for each patient. Disturbances were considered "mild" if, after temporary decrease in the PAS dose, they did not interfere

[3] Test preparations were provided by Dr. A. Wander S. A., Berne, Switzerland.

with continuation of the treatment. Disturbances were called "severe" if it was necessary to discontinue the PAS preparation then being used. At the beginning, midway, and at the end of the treatment, urine was tested for albumin, urobilinogen, and sediment. Statistical evaluation of the findings was carried out with the aid of the 2×2 contingency table and the chi-square test. Dosage schedules were not evaluated separately because nearly all of the patients treated (87 per cent) weighed up to 70 kg. and therefore received, in accordance with the procedure outlined above, daily doses of 12 gm. of either one of the PAS preparations.

Results

Of 175 patients, 85 started the test with benzoyl-PAS, and 90, with PAS. Fifteen of the B-PAS group and 10 of the PAS group were eliminated for various reasons (table 1). Thus, 70 patients of the B-PAS group and 80 of the PAS group were available for assessment.

Sixty-three (90 per cent) of the patients treated with B-PAS and 50 (63 per cent) of those treated with PAS completed the twelve-week trial (table 2). Early discontinuation of the treatment due to serious disturbance was required in 7 cases (10 per cent) of the B-PAS group and in 30 cases (37 per cent) of the PAS group. The difference in the rate of incidence is statistically significant ($P < 0.001$). This shows that it was possible to complete the B-PAS treatment in a significantly higher percentage of patients, or that the incidence of serious disturbances under B-PAS therapy was significantly smaller (figure 1). Likewise, symptomless cases under B-PAS therapy were significantly more frequent (51, or 73 per cent) than under PAS therapy (35, or 44 per cent). The incidence of mild disturbances was about the same for both products (17 per cent with B-PAS and 19 per cent with PAS).

In 21 patients B-PAS was substituted for PAS because of PAS intolerance, and in 2 patients PAS was substituted for B-PAS because of B-PAS intolerance. Sixteen of the 21 patients of the first group completed their treatment, 12 of them without untoward symptoms. Of the 2 patients

TABLE 1

Summary of the Number of Cases Observed in Tolerance Studies

	Patients Treated With B-PAS	Patients Treated With PAS
Treatment completed	63	50
Treatment altered due to severe disturbances:		
Discontinued altogether	5	6
Changed over to the other preparation	2	24*
Total of assessable cases	70	80
Nonassessable cases:		
Left hospital	8	3
Medical reasons	4	6
Poor collaboration	3	1
Total number of patients investigated	85	90

* Two of these patients left the hospital after changing over to B-PAS; one case was discontinued for medical reasons.

TABLE 2

Tolerability of B-PAS and PAS: Results for the Assessable Cases

	Patients Treated With B-PAS						Patients Treated With PAS					
	Treated First With B-PAS		Changed Over to B-PAS, Due to PAS Intolerance		Total		Treated First With PAS		Changed Over to PAS Due to B-PAS Intolerance	Total		
	Number	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*	
Treatment completed	63	90	16	76	79	87	50	63	1	51	62	
No disturbances	51	73	12	57	63	69	35	44	1	36	44	
Mild disturbances	12	17	4	19	16	18	15	19	0	15	18	
Treatment discontinued due to severe disturbances	7	10	5	24	12	13	30	37	1	31	38	
Total	70	100	21	100	91	100	80	100	2	82	100	

intolerant to B-PAS, one was able to complete treatment after a changeover to PAS.

The side effects, which were similar with both preparations, occurred mainly during the first three weeks. The most frequent disturbances were the well-known intestinal disorders, i.e., "heartburn," a sense of pressure, nausea, vomiting, loss of appetite, flatulence, gastric cramps, and occasional diarrheas (table 3). Under PAS therapy, these disturbances were significantly more frequent ($P < 0.001$), and in some instances it was necessary to discontinue therapy. Second in importance were allergic reactions such as itching and urticarial rashes; they were seen more often with PAS, and it was only with this drug that medication had to be completely discontinued. A third group of about the same number of patients exhibited uncharacteristic symptoms, such as aversion to the drugs or vertigo. The incidence of these disturbances was about the same for both preparations.

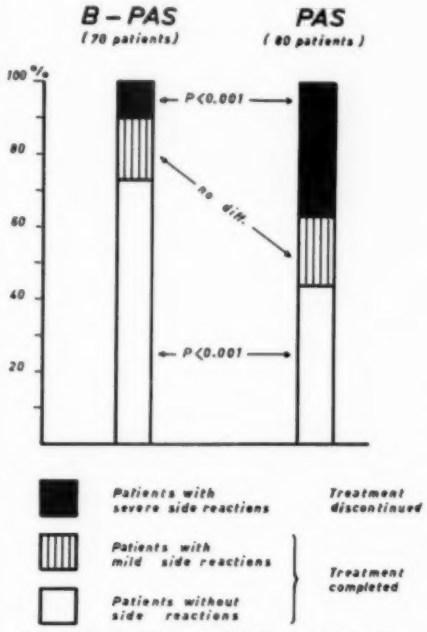

FIG. 1. Tolerability of B-PAS and of PAS.

The urinary findings of the whole test series were insignificant with the exception of 2 patients withdrawn from the tolerance test, in whom an increase in urobilinogen was found. One patient had an attack of cholecystitis under B-PAS therapy; the other, under PAS therapy, a relapse of hepatitis following an earlier treatment with pyrazinamide.

DISCUSSION

When investigating the tolerability of a medication such as PAS, the statements of the patients are depended upon to a large extent. For reliable results, as many extraneous factors as possible which might influence the experimental findings must be eliminated. For this reason, it was endeavored to conduct this study in such a manner as to exclude the influence on the final results of such factors as taste, pharmaceutical form, dosage, kind of salt used, the tuberculostatic agent employed in multiple-drug treatment, general hospital care, and the conscious or unconscious attitude of the patients, nursing personnel, and investigators. The double blind methodology shows how the elimination of these factors of bias was attempted, to permit, as far as possible, consideration of the administration of PAS or benzoyl-PAS as the only variable factor in tolerability.

In order to arrive at statistically assessable results in the course of one year, the test period has been limited to twelve weeks; on the other

TABLE 3

INCIDENCE AND TYPES OF SIDE EFFECTS DURING TREATMENT WITH B-PAS AND WITH PAS

Side Effects	Treatment With B-PAS				Treatment With PAS			
	Treatment Completed		Treatment Discontinued		Treatment Completed		Treatment Discontinued	
	Number of Episodes	*Number of Patients*	*Number of Episodes*	*Number of Patients*	*Number of Episodes*	*Number of Patients*	*Number of Episodes*	*Number of Patients*
Intestinal disturbances	19	19	13	12	24	18	46	40
Allergic reactions	3	2			1	1	5	4
Uncharacteristic disturbances	2	2	2	2	4	2	4	4

hand, as many patients as possible have been included. The fact that the relatively short test period still provides a sufficiently reliable basis for evaluating the side effects is shown by the investigations of Phillips (12). This investigator found that, with a period of observation of one year, the incidence of side effects due to calcium B-PAS was twice as high during the first four months as during the second four-month period, while practically no disturbances were recorded thereafter; with reference to PAS, these proportions were still more apparent. In the trial being reported here, the side effects were observed chiefly during the first three weeks.

With respect to the comparability of the two groups, they have been checked according to age and sex distribution. The average age of the two groups was found to be approximately the same (39.5 years for the B-PAS group and 37.5 years for the PAS group). In both groups, the female patients predominated, more in the PAS group (50 females, 30 males) than in the B-PAS group (37 females, 35 males). This discrepancy, discovered belatedly, suggested the need to calculate separately for the male and for the female patients the differences in the rate of serious disturbances due to B-PAS and to PAS. Due to marked disturbances, the medication had to be discontinued in 9 per cent of male patients under B-PAS therapy and in 30 per cent of male patients under PAS therapy. The difference is slightly smaller for the male patients, and somewhat greater for the female patients than that recorded in table 2 for all assessable cases. With the smaller numbers, the statistical significance is correspondingly less. However, the over-all results are not appreciably modified by the unequal distribution of the female patients in the two groups.

With respect to calcium PAS, the usual therapeutic dosage was employed, while the dosage of calcium B-PAS was higher than that usually administered (7, 9). Indeed, this latter compound was dosed on the basis of its B-PAS content, and not as a compound per se. Nevertheless, the tolerability of B-PAS was found to be significantly superior: with this drug 90 per cent of the patients completed their treatment without any or with only slight disturbances, while this occurred in slightly less than two-thirds of the patients treated with PAS.

It was not possible to compare how often one PAS preparation could be substituted for the other. For replacing PAS with B-PAS, 30 patients were available, while only 7 were available for the opposite substitution. In both groups, side effects of such intensity occurred in several cases that no further PAS therapy could be imposed upon these patients. For this reason, only 2 patients with B-PAS intolerance could be switched to PAS, a figure which precludes any inference as to the success of the drug substitution in the presence of B-PAS intolerance.

Among the 30 patients with severe side effects due to PAS therapy, an attempt at changing over to B-PAS could be made in 21 cases. Sixteen of these completed their treatment. While the number of patients in this group is small, calculation of probabilities allows the prediction that at least 49 per cent, and at most 90 per cent, of patients intolerant to PAS are able to continue their treatment with B-PAS ($P = 0.05$). This is consistent with the statements of Steinlin (8) and Phillips (13), that about two-thirds of patients intolerant to PAS can be further treated with B-PAS.

The over-all incidence of disturbances due to PAS therapy observed in this study is relatively high, about twice as high as that observed by Phillips (12), but matches approximately that reported by Ståhle (7) (table 4). Likewise, the much lower rate of disturbances during B-PAS therapy is expressed in the figures reported by Ståhle and in this paper, but not in those reported by Phillips. In the present study, B-PAS medication had to be discontinued in about the same proportion as that stated by Phillips, while for PAS the proportion was much greater. Neither of the PAS preparations had to be discontinued by Ståhle during the three-month period. The difference in the results of these three trials cannot presently be explained.

While, on the whole, the types of side effects due to B-PAS were consistent with those reported by Phillips (12, 13), no fever, pruritus, and dysuria in connection with B-PAS therapy were seen. Allergic skin reactions (itching, urticaria) were observed with both medications (*see* table 3), but the symptoms were much more intense under PAS therapy. In 4 patients, PAS medication had to be stopped for this reason, and it was considered inadvisable to continue treatment with B-PAS as Phillips and associates (9) had successfully done in three of their own cases.

TABLE 4

COMPARISON OF THE INCIDENCE OF SIDE EFFECTS DURING B-PAS AND PAS TREATMENT ACCORDING TO DIFFERENT INVESTIGATORS

	Ståhle (1957) (Three Months)				Phillips (1956) (Four Months)				Present Trial (Twelve Weeks)			
	Calcium B-PAS		PAS		Calcium B-PAS		Sodium PAS		Calcium B-PAS		Calcium PAS	
	Number	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*	*Number*	*Per Cent*
Patients without disturbances	23	88	13	54	38	64	42	74	51	73	35	44
All patients with disturbances	3	12	11	46	21	36	15	26	19	27	45	56
Treatment discontinued	—		—		8	14	9	16	7	10	30	37
Total	26		24		59		57		70		80	

This investigation is believed to have shown that B-PAS is significantly better tolerated than PAS. According to modern concepts (14), every known patient with pulmonary tuberculosis should receive antituberculosis therapy for at least six months beyond the disappearance of tubercle bacilli from the sputum and, on the whole, for at least one year uninterruptedly. Combined treatment with isoniazid and PAS is considered standard despite the difficulties due to the limited tolerability of PAS (15). This view is based upon the reliable delay in the emergence of drug-resistant strains of tubercle bacilli, the possible effects of PAS on the isoniazid metabolism, and the excellent therapeutic effect of this drug combination, apart from the fact that both drugs can be given by mouth (15). A PAS preparation such as calcium B-PAS, capable of producing the same effects as PAS in multiple-drug therapy while being significantly better tolerated, thus deserves special consideration as a companion drug for long-term treatment of tuberculosis. Whenever PAS meets with intolerance, an attempt should be made to replace it with B-PAS so that the majority of patients may be able to continue the reliable combined isoniazid therapy.

SUMMARY

In a double blind test lasting twelve weeks, the tolerability of the calcium salt of benzoyl-PAS (B-PAS) has been compared with that of PAS in 150 patients with pulmonary tuberculosis.

Ninety per cent of the patients treated with B-PAS and 63 per cent of those treated with PAS were able to complete their treatment; 10 per cent of the patients treated with B-PAS and 37 per cent of those treated with PAS had to discontinue the treatment because of marked disturbances—particularly gastrointestinal disorders. The difference of incidences is highly significant. The rate of mild side effects which did not interfere with the continuation of the treatment was the same for both preparations (17 per cent and 19 per cent, respectively).

Of 21 patients intolerant to PAS, 16 were able to complete their treatment with B-PAS.

The importance of the better tolerability of B-PAS for modern long-term treatment is emphasized.

SUMARIO

La Tolerabilidad del Benzoilo-PAS Cálcico y del PAS Cálcico: Estudio "Double Blind"

En un estudio "double blind" que duró doce semanas, se comparó la tolerabilidad de la sal cálcica del benzoilo-PAS (B-PAS) con la del PAS en 150 enfermos de tuberculosis pulmonar.

Un 90 por ciento de los enfermos tratados con B-PAS y un 63 por ciento de los tratados con PAS pudieron terminar su tratamiento; un 10 por ciento de los tratados con B-PAS y un 37 por ciento de los tratados con PAS tuvieron que abandonar el tratamiento debido a perturbaciones acentuadas, en particular trastornos gastrointestinales. La diferencia en las incidencias es sumamente significativa. El coeficiente de leves efectos colaterales que no impidieron continuar el tratamiento fué casi idéntico para ambas preparaciones (17 y 19 por ciento, respectivamente).

De 21 enfermos intolerantes al PAS, 16 pudieron terminar el tratamiento con B-PAS.

Recálcase la importancia que reviste la mejor tolerabilidad al B-PAS para el moderno tratamiento a largo plazo.

Resume

La tolérance pour le Calcium Benzoyle-PAS et le Calcium PAS: Etude "double blind"

Dans une épreuve "double blind" durant douze semaines, la tolérance pour le sel de calcium de benzoyle-PAS (B-PAS) a été confronté avec celle du PAS chez 150 patients atteints de tuberculose pulmonaire.

Quatre-vingt-dix pour cent des patients traités par le B-PAS et 63% de ceux traités par le PAS ont pu achever leur traitement; chez 10% des malades traités par le B-PAS et 37% de ceux traités par le PAS, le traitement a dû être discontinué en raison de la présence de réactions secondaires marquées—en particulier de troubles gastro-intestinaux. La différence entre les incidences est extrêmement significative. Le taux des réactions secondaires modérées ne prévenant pas la poursuite du traitement était le même pour les deux préparations (respectivement 17% et 19%).

Parmi 21 patients intolérants au PAS, 16 ont pu achever leur traitement avec le B-PAS. L'importance d'une meilleure tolérance pour le B-PAS dans le traitement moderne à long terme est soulignée.

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Case Reports

MUCORMYCOSIS OF THE LUNG[1]

A Case Without Significant Predisposing Factor

HERMAN W. BLANKENBERG AND DIRK VERHOEFF

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Introduction

Mucormycosis is a usually fatal fungal infection often caused by the *Phycomycetes rhizopus* and, as a rule, is seen concomitant with severe debilitating disease (often uncontrolled diabetes). Recently two cases of this disease involving the central nervous system and one case of mucormycosis of the bronchus have been reported in which the patient survived after removal of the focus (1–3).

The patient of the present case survived after a right lower and middle lobectomy and the case is presented because, at the time when the mucormycosis was manifest, no evidence of diabetes was found. One year later, however, glucosuria was discovered.

Case Report

E. E. G. was a 23-year-old Negro female who was admitted to Mecklenburg Sanatorium on November 5, 1956. Her health apparently had been good, although she had had several hospital admissions for so-called painful and profuse menstruation. Three months before admission she developed a nonproductive cough. No fever was noted. Two months later she developed what she described as a chest cold. She finally called her physician one week thereafter who gave her one dose of penicillin. Ten days before admission the patient began to produce about one ounce of blood-streaked sputum daily and she developed a fever. She was admitted to another hospital and for three days was treated with penicillin. Since her chest roentgenogram showed a large pulmonary defect, she was transferred to this hospital.

On admission she appeared acutely ill, somewhat confused mentally, with a high fluctuating temperature and marked dehydration. On examination coarse moist rales were noted in the right chest, with decreased breath sounds and dullness over the right base. The right diaphragm did not move. The heart appeared slightly enlarged. The remainder of the physical findings were entirely within normal limits.

Roentgenographic and laboratory findings: The hemoglobin was 7.5 mg.; there were 2,600,000 erythrocytes per cu. mm. and 17,700 leukocytes, with 94 per cent polymorphonuclears, 5 per cent lymphocytes, and 1 per cent monocytes. The urine was negative for albumin and sugar, but showed numerous pus cells. The fasting blood sugar was 83 mg. per 100 ml. The electrocardiogram showed tachycardia, right axis deviation, and inversion of T waves, indicating myocardial damage. Chest roentgenograms suggested the presence of a large pulmonary abscess or cyst with multiple fluid levels in the right lower lobe. A gastrointestinal series failed to show a connection of this lesion with a subdiaphragmatic structure or esophagus. Bronchoscopy revealed obstruction of the lower lobe bronchi. Bronchograms failed to show a connection between the lesion and the bronchial tree, but a roentgenogram taken the next day showed some iodized oil in the cavity. Several nodular densities were noted in the left lung field. The largest, 2 cm. in diameter, was at the level of the second anterior interspace (figure 1). Culture of the sputum revealed the presence of a mixed flora, predominantly hemolytic *Staphylococcus aureus*, gamma Streptococcus, and *Diplococcus pneumoniae*. No fungal cultures were made. Smears failed to show tubercle bacilli, and the tuberculin skin test, 1:1,000, was negative.

As the predominating organisms were susceptible to chloramphenicol and erythromycin, the patient was started on intramuscular injections of erythromycin. Her anemia was corrected and she was hydrated with intravenous fluids. Also, sulfisoxazole was given for the pyuria. Notwithstanding these measures, her temperature remained between 104° and 105°F. Following bronchoscopy she expectorated large amounts of purulent material.

A right thoracotomy was performed on November 14, 1956. The middle and lower lobes were found to be necrotic and were removed (figure 2). Following operation, intravenous chloramphenicol and intramuscular erythromycin injections were given. Her temperature dropped immediately to 100°F. and was normal a few days later.

Macroscopic examination: Macroscopic inspection of the removed lobes revealed large ill-defined cavities in the lower lobe measuring 6 by 5 by 4 cm., and in the middle lobe, 4 by 4 by 4 cm. The cavities were surrounded by thick layers of grayish-brown fibrinous hemorrhagic material and thick layers of indurated whitish-gray necrotic

[1] From Mecklenburg Sanatorium, Huntersville, North Carolina.

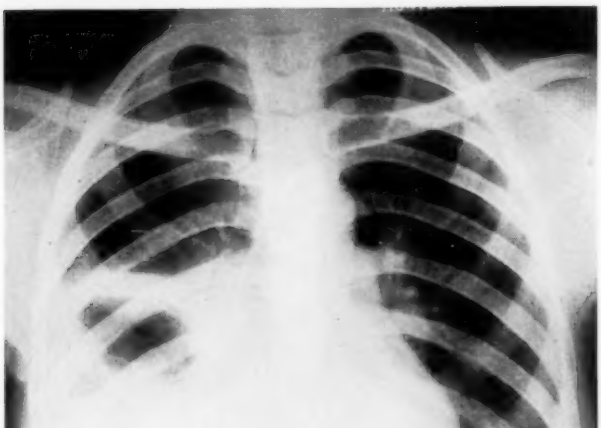

FIG. 1. Postero-anterior roentgenogram of November 6, 1956. Large pulmonary abscess at right base. Note the nodular density in the second anterior interspace, left.

FIG. 2. Right middle lower lobe showing multiloculated abscess.

tissue. Many branches of the medium-sized bronchi were completely obliterated, filled with necrotic whitish-brown firm material. Some of the bronchi had continuity with the cavities. In one area near the diaphragmatic surface, the bronchi were suddenly markedly enlarged in caliber and the wall was ulcerated and covered by yellowish-gray fibrinous material. Cross-sections of the lung showed many small minute foci of yellowish-gray, firm, and soft friable areas near the large cavity.

Microscopic examination: The examination showed areas of liquefaction necrosis surrounded by thick layers of eosinophilic, amorphous, or granular material with mild infiltration of neutrophils, eosinophils, lymphocytes, and plasma cells. Many multinucleated foreign-body giant cells were seen. In the necrotic area numerous basophilic, irregularly shaped structures were observed. Around the necrotic spaces many large blood vessels and bronchi showed almost complete necrosis of the wall. Other blood vessels showed hyalinization of the wall or recent thrombus of the lumen, characterized by obliteration with spindle-shaped cells with hyperchromatic large nuclei. Occasionally even small bronchi contained much foreign material. The foreign material is characterized by broad branching coenocytic hyphae of varying thickness. When the fungus appeared to be growing luxuriantly, it was thick; in regions in which infarction had occurred or the tissues were necrotic, hyphae were shrunken and irregular. A marked tendency to invade vessels was noted (figure 3).

After the pathologic diagnosis was established, all antimicrobial drugs were discontinued and the patient was treated with sulfisoxazole and nystatin.

It should be noted that the pulmonary density (*see* figure 1), located in the second anterior interspace on the left, had increased in size on November 12, 1956 (figure 4), showing a center of increased radiolucency. It was suspected of being a beginning metastatic abscess. Following surgery this density and other smaller ones (not so easily visible on the reproduction) gradually disappeared. On November 25, 1956, only a minimal residual scar remained, and on January 10, 1957, the left lung had cleared completely. The heart size and electrocardiographic tracings also had returned to normal at that time. Throughout her hospital stay the patient's urine failed to show reducing substances. Several blood sugars were within normal limits. She gained 13 pounds while in the hospital and maintained her weight after discharge. Follow-up observation through December, 1957 (one year after operation), showed the patient to be in good health with a normal roentgenogram and a normal electrocardiogram. Recently her family physician reported that in January, 1958, he had noted reducing substances in her urine; later she was admitted to another hospital where elevated fasting blood sugar, ranging up to 287 mg. per 100 ml., was found. She had mild acidosis, and later her diabetes was controlled satisfactorily with 20 units of Lente insulin and a 2,100-calorie diet.

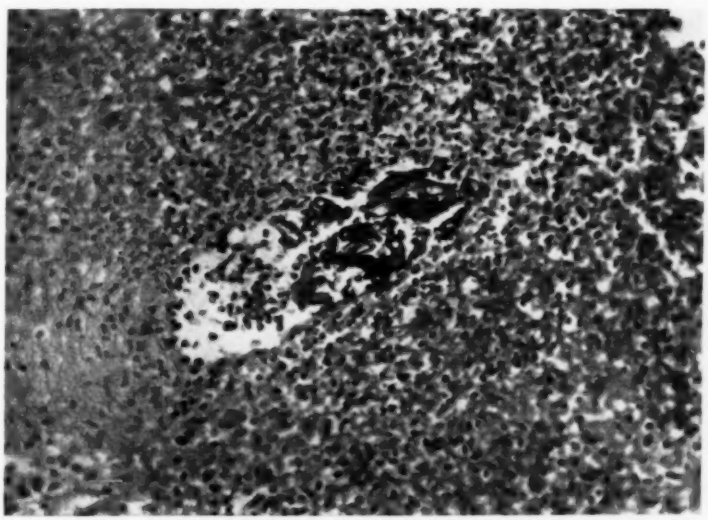

FIG. 3. *Phycomycetes rhizopus* in lung. Infiltrating in blood vessel.

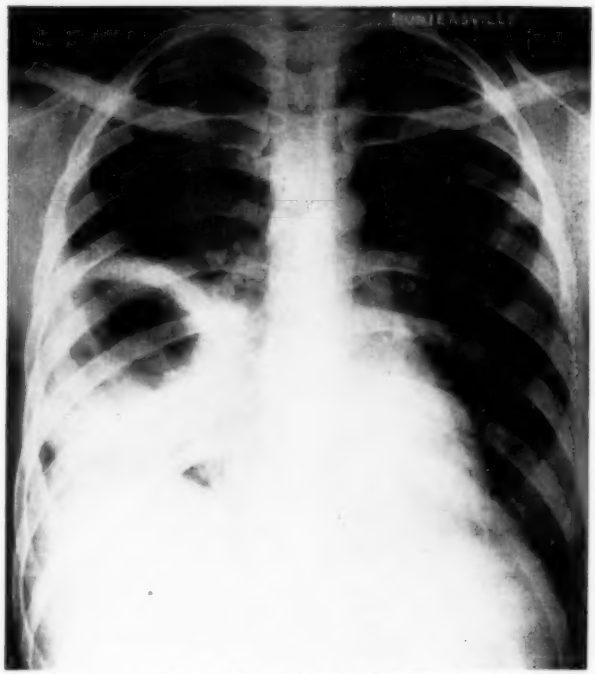

FIG. 4. Postero-anterior roentgenogram of November 12, 1956. The density in the left second anterior interspace shows a center of increased radiolucency.

DISCUSSION

Many writers have commented on the generally debilitated condition of persons infected with mucormycosis. Paltauf (4) reported the case of a patient who had severe diarrhea, vomiting, and jaundice. Twelve other patients with central nervous system manifestations had uncontrollable diabetes with usually severe acidosis. One patient was an infant two and a half months old with a two-week history of severe diarrhea.

Of 16 cases of pulmonary involvement, five patients had widespread carcinoma or leukemia; three had diabetes; two, severe diarrhea; one, tuberculosis; and one was severely burned. In three others no specific disease was mentioned. In nine cases the hemoglobin level was reported, and in only three of these patients the level was in the low normal range; all others had marked anemia. The patient of the present case was anemic, but thorough investigation failed to show any associated disease at the time she was being treated by the writers. It is interesting that now she has developed a mild diabetes.

The pathogenesis of the abscess in pulmonary mucormycosis is one of fungal invasion of the wall of the pulmonary vessels with subsequent thrombosis, producing an infarct in which the fungus reproduces rapidly. The fungus then may spread hematogenously or intrabronchially to other parts of the body (5, 6). In the central nervous system disease, the pathogenesis seems to be similar, and the paranasal sinuses are the primary focus of infection (1, 2, 7). It appears, therefore, that surgical removal of the infected and necrotic tissue is indicated as soon as the patient's condition permits. This seems to have been the major factor in the recovery of the patient of the present case.

The patient responded immediately to the removal of the major focus and continued to improve thereafter. Antimicrobial therapy was stopped after the diagnosis was made because of the ineffectiveness reported in the literature and the known tendency to favor growth of certain fungi. Nystatin was then started on a trial basis. Perhaps iodides would have been beneficial, but they were not used since the patient was improving on the previously outlined therapy.

Summary

A case is reported of a patient who survived following removal of the major focus in the lung. The patient's survival is attributed to four important factors: early surgical removal of the major pulmonary focus; prompt institution of general supportive measures; no significant complicating disease in a young individual (diabetes appeared one year after discharge); and withdrawal of "broad-spectrum" antimicrobials.

Sumario

Mucormicosis del Pulmón. Un Caso sin Factor Predisponente de Importancia

Preséntase el caso de un enfermo con mucormicosis que sobrevivió a continuación de la extirpación del foco principal en el pulmón. Se atribuye la sobrevivencia del sujeto a cuatro factores importantes: temprana eliminación quirúrgica del foco pulmonar mayor; pronta iniciación de medidas analépticas generales; no existir ninguna importante enfermedad como complicación en un individuo joven (al año del alta apareció diabetes); supresión de los antimicrobianos de espectro ancho.

Resume

Mucormycose du poumon. Un cas sans facteur prédisposant significatif

Présentation d'une observation de malade ayant survécu à l'excision du foyer pulmonaire majeur. La survie du malade est attribuée à quatre facteurs importants: excision précoce du foyer pulmonaire majeur; prompte instauration de la thérapeutique de soutien; absence de maladie associée significative chez un sujet jeune (apparition d'un diabète un an après la sortie de l'hôpital); suppression des antibiotiques à large spectre antibactérien.

Acknowledgment

The writers wish to thank Dr. Paul Kimmelstiel and Dr. Gordon D. Lazerte of the Department of Pathology of the Charlotte Memorial Hospital for their help in rendering the diagnosis, and Dr. Roger Baker of the Veterans Administration Hospital, Durham, North Carolina, for reviewing the microscopic slides. Dr. Baker adds the following note:

"The hyphae are characteristic of those seen in pulmonary mucormycosis, and the characteristic thrombosis of vessels and infarction are present. There is, in addition, abscess formation which I did not see in the cases of pulmonary mucormycosis which I reported. I do not see bacterial masses in this H. & E. stain, and presume that the abscesses have been caused by the fungus, or represent liquefaction of the infarct."

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NONREACTIVE TUBERCULOSIS[1]

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INTRODUCTION

Landouzy (1–3), in 1882, described for the first time a form of tuberculosis which was clinically similar to typhoid fever, which he called typhobacillosis. New cases were presented by Gougerot (4), a disciple of Landouzy, in 1908. The phases of the typhobacillosis were apparently experimentally reproduced in rabbits by Yersin (5) in 1888 and by Gougerot (6) in 1908. According to Siegmund's review of 1939 (7), in the German literature this process was called "sepsis tuberkulosa acutissima," "sepsis tuberkulosa gravissima," "tuberkulosepsis," and "typhotuberkulose." Siegmund himself introduced the name "generalized nonreactive tuberculosis." The term "acute caseating miliary tuberculosis" was used by Rich and McCordock (8) in 1929 for this condition, and O'Brien (9), in his exhaustive review of 1954, suggested "nonreactive tuberculosis." Among the Latin American countries this type of tuberculosis is known as Landouzy's typhobacillosis.

The most common clinical course is that of an acute overwhelming infection (7, 9–11). A disturbance of the hematopoietic system (7, 9, 10) is frequently present during life, consisting of anemia, polycythemia, agranulocytosis, leukemoid reaction, or thrombocytopenia. According to O'Brien (9), a pre-existing hematologic disease (such as leukemia or myelosclerosis) is found in about 27 per cent of the cases. The blood picture was normal in only six of the sixty-six cases collected by O'Brien. Splenomegaly is nearly always found, and hepatomegaly often. Pathologically, as was emphasized by the German school, miliary foci of necrosis surrounded by normal parenchymal cells are found in many organs. Foci up to 1 cm. in diameter have been reported (9). There is no development of tuberculous granulation tissue, and occasionally a poor cellular response is noted. The lesions contain enormous numbers of acid-fast bacilli. O'Brien (9) found that tubercle bacilli were identified by adequate cultural methods and animal inoculation in 18 of the 66 reviewed cases; in 13 the strains were of human origin, and in 5 of bovine origin. Rich (12) has denied, with good reason, the role of the avian type of tubercle bacilli, which was considered to be the etiologic agent in the older literature.

In recent years, numerous reports have appeared dealing with the deleterious effect of adrenocortical steroid therapy in tuberculosis. In experimental tuberculosis it has been observed that cortisone induces a poor cellular response and a definitely unfavorable course (13–16), adverse results having been also reported when corticotropin was used (17). In man, reports of development (18–24), aggravation (22), and reactivation (25) of tuberculosis following corticotropin and/or cortisone therapy have also been made. In one of these reports (24), a poor cellular response was found. O'Brien (9) has stated that, in some patients treated with cortisone for a different disease, an unsuspected tuberculous process has advanced rapidly, the histology of the lesions being strikingly like that found in naturally occurring nonreactive tuberculosis. Last year, a report of development of a fatal widespread tuberculosis following a fourteen-month period of steroid therapy in a patient with disseminated lupus erythematosus appeared in the literature (26). During twelve of these months the treatment consisted of prednisone, and the microscopic studies revealed a very poor cellular response.

The present case report of nonreactive tuberculosis is of interest in that it originated in a patient with known chronic lymphatic leukemia to whom steroid therapy was given. As may be seen, the administered therapy was not an obstacle in the final interpretation.

CASE REPORT

A 72-year-old, white, male, retired carpenter was admitted to the M. D. Anderson Hospital of Houston, Texas, on August 13, 1957, because of a febrile condition of unknown etiology associated with chronic lymphatic leukemia.

When in February, 1957, the patient was ad-

[1] From the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

mitted to a hospital in Texas for excision of a recurrent basal cell carcinoma of the right temple and subsequent grafting, a routine blood count showed 70,000 leukocytes per cu. mm., with 98 per cent mature lymphocytes. Symptoms or signs of leukemia were absent. Repeated blood counts over a month disclosed no appreciable change of these figures.

In early July, 1957, the patient developed a "cold" with progressive cough, and five days later his temperature rose to 102°F. The fever persisted and the patient was admitted to another hospital on July 17, 1957. Roentgenograms of the chest showed minimal infiltration of some type in the left upper lobe as well as emphysema. The peripheral blood count remained unchanged. Sputum was not produced. Penicillin and erythromycin were given, and the patient was discharged with a cough on July 23, 1957.

Two days later he was readmitted to the first hospital because of chills and temperature elevation up to 103°F. A small "shotty" left posteroinferior cervical node was found, and the liver edge was one finger-breadth below the right costal margin. The leukocyte count was unchanged, and a urinalysis showed 1-plus albumin. The roentgenograms of the chest exhibited clear lung fields. The patient continued to have fever up to 103–104°F. The elevation of temperature was diurnal with peaks at 6 or 7 a.m. and 4 or 7 p.m. and was usually preceded by frank chill. The patient produced no sputum. Malaria smears and agglutination reactions for several infections were negative. He received corticotropin in the form of Acthar Gel® (40 units daily) on July 27, 28, and 29, 1957. On August 10, a few petechiae appeared on the anterior aspect of the thorax and the liver edge was two finger-breadths below the right costal margin. There was a question of tenderness of the liver edge. Starting on August 3, 1957, and for five days, 5 mg. of prednisone were given four times a day, without any appreciable change in the clinical condition. On August 8, 1957, cortisone was started along with 6-mercaptopurine. Three hundred milligrams of cortisone were given the first day, 200 mg. the second day, and 150 mg. daily on the third, fourth, and fifth days. One hundred milligrams of 6-mercaptopurine were administered daily during the same five-day period. On August 13, 1957, the patient was transferred to the M. D. Anderson Hospital for further diagnostic procedures and treatment.

The significant physical findings were: an elderly white male in fair general condition, a skin graft without hair on the right side of the scalp, a necrotic area just above each maxillary region, a heavily coated tongue, fine rales and decreased resonance at the base of the lungs, and an enlarged liver. The liver edge was 3 cm. below the right costal margin. The blood pressure was 130 systolic, 50 diastolic in mm. of mercury, pulse 104, respirations 30, temperature 104°F.

Leukocytes were 67,450 per cu. mm. and the differential leukocytic count showed 98 per cent lymphocytes. The platelet count was 45,000 per cu. mm. These tests were repeated once, with the following results: 91,400 leukocytes per cu. mm., 99 per cent lymphocytes, and 24,000 platelets per cu. mm. Hemoglobin was 10.2 gm. per 100 ml. A urinalysis showed 2-plus albumin, 1.022 specific gravity, 10 to 12 leukocytes, and numerous granular casts. A routine culture of urine was negative. Urea nitrogen was 31 mg. per 100 ml. Liver function studies revealed a 3-plus cephalin flocculation; a bromsulphalein retention of 28 per cent; thymol turbidity was 3.33 units, alkaline phosphatase was 3.7 Bodansky units per 100 ml.; serum bilirubin was 5.52 mg. per 100 ml. Total serum proteins were 4.3 gm. per 100 ml., with an albumin fraction of 3.1 gm. per 100 ml. Many acid-fast bacilli were found in the sputum smears on August 16, 1957, and, using the Löwenstein-Jensen medium, a luxuriant growth of acid-fast organisms typical of *Mycobacterium tuberculosis* was obtained from the sputum three weeks later. Smears and cultures of the sputum were negative for fungi. Skin tests with tuberculin (first-strength PPD), blastomycin, coccidioidin, and histoplasmin were negative. The roentgenograms of the chest showed diffuse nodular infiltration throughout both lung fields, heaviest at the hilar levels. A submitted film of the chest dated July 26, 1957, disclosed the lung fields to be clear.

The patient followed a febrile course, the temperature varying from 100° to 104°F.; on only one occasion was it 99°F. On August 16, 1957, jaundice was noted, which gradually became more marked. The patient died on August 20, 1957. During August 16 and 17, isoniazid (100 mg. three times daily), PAS (4 gm. three times daily), and streptomycin (1 gm. every six hours) were given. One gram of streptomycin was given, daily, on August 18 and 19.

Post-mortem Examination

The skin and mucosa were icteric. Small scattered purpuric areas were present throughout the body. The lungs showed subpleural hemorrhages. All of the pulmonary lobes were markedly hyperemic and contained numerous yellowish fairly rounded caseonecrotic submiliary, miliary, and bronchopneumonic lesions ranging in size from pin-point to 0.5 cm.; they were confluent and in greater number in the lower lobes. The right lung weighed 1,000 gm.; the left, 930 gm. The bronchopulmonary, tracheobronchial, and paratracheal lymph nodes were enlarged and of rubbery consistency; they ranged from 1 to 5 cm. in their major dimensions. The largest lymph node was a right lower tracheobronchial one. All of these lymph nodes were anthracotic and contained yellowish-greenish caseonecrotic confluent areas. These same changes were also found in the lymph nodes of the venous angles.

Post-mortem roentgenograms of the freshly removed lungs were not obtained.

The heart was somewhat flabby and weighed

350 gm. The liver weighed 2,030 gm. and, on section, it showed minute soft yellow areas throughout. The spleen weighed 400 gm. and contained numerous irregular yellowish caseonecrotic areas throughout, ranging from 0.1 to 0.3 cm. in diameter. The examination of the thyroid, parathyroids, small intestine, large intestine, appendix, pancreas, adrenals, kidneys, excretory urinary tract, prostate gland, and seminal vesicles revealed nothing remarkable except a few small cortical scars in the kidneys. The axillary, hepatic, gastric, peri-aortic, and pelvic lymph nodes were slightly enlarged, had a rubbery consistency, and exhibited homogeneous shiny gray cut surfaces. The pancreatico-lienal lymph nodes ranged from 1 to 2 cm. in their major dimensions, were somewhat firm, and contained lesions similar to those seen in the intrathoracic lymph nodes already described. The brain showed only slight engorgement of the meningeal vessels over the gyri; it weighed 1,400 gm. The marrow of the sternum and bodies of the thoracic and lumbar vertebrae was thick and reddish-gray. The tonsils and nasopharynx were not examined.

Microscopic Studies

Lungs: Bronchopneumonic, miliary, and miliary-pneumonic necrotic lesions were noted here and there. They were of irregular morphology, had an abundant amount of nuclear debris, and were abruptly demarcated from the surrounding pulmonary parenchyma. Early caseation was present only in some of them. The lesions were mainly of the bronchopneumonic and miliary types. Apparently, most of the nuclear debris came from mononuclear cells and only some from polymorphonuclear leukocytes. There was neither granulation tissue, epithelioid cells, Langhans' giant cells, nor fibrosis. In between the lesions, the lung parenchyma exhibited marked recent hyperemia, hemorrhagic alveolitis, alveolar edema, and frequent intra-alveolar desquamated swollen alveolar cells. The hemorrhagic alveolitis was more marked in the lower lobes (figure 1). The necrotic lesions generally displayed remnants of elastic fibers, and only very rarely was a fairly well-preserved pneumonic pattern detected. The apices of the lungs disclosed collapso-induration. The necrotic lesions appeared massively filled with acid-fast bacilli which were of medium length and thickness, finely granular, well stained, straight or slightly curved, and were isolated or formed small clumps. There were relatively few short and long bacilli. In the alveoli close to the lesions, about 80 bacilli per field were counted, all of them extracellular. The perifocal lesions showed only scanty extracellular septal and intra-alveolar bacilli. The acid-fast bacilli were always well preserved; degenerative forms were not found in either the necrotic foci or the perifocal lesions. These bacilli are similar in

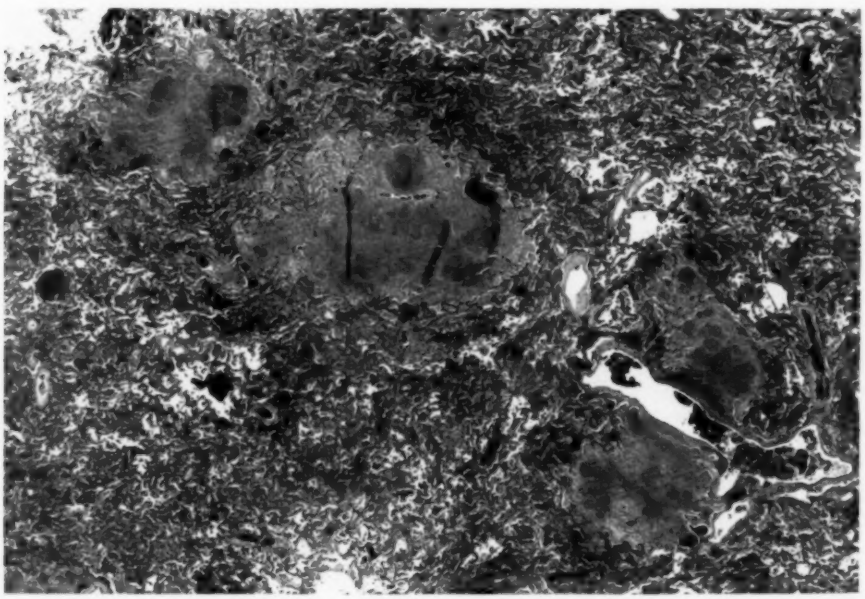

FIG. 1. Lung: Caseonecrotic lesions fairly well demarcated from the pulmonary parenchyma and containing cellular debris; hemorrhagic alveolitis is present between them. Epithelioid and Langhans' giant cells are not present. (Hematoxylin and eosin stain, × 160)

their morphologic characteristics and preservation to those observed in the tracheobronchial and paratracheal lymph nodes, liver, and spleen.

Tracheobronchial and paratracheal lymph nodes: Tracheobronchial and paratracheal lymph nodes were mostly replaced by large irregular confluent necrotic areas containing an abundant amount of nuclear debris. This nuclear debris looked like that seen in the necrotic lesions of the lungs. Some elongated cells which suggested fibroblasts were

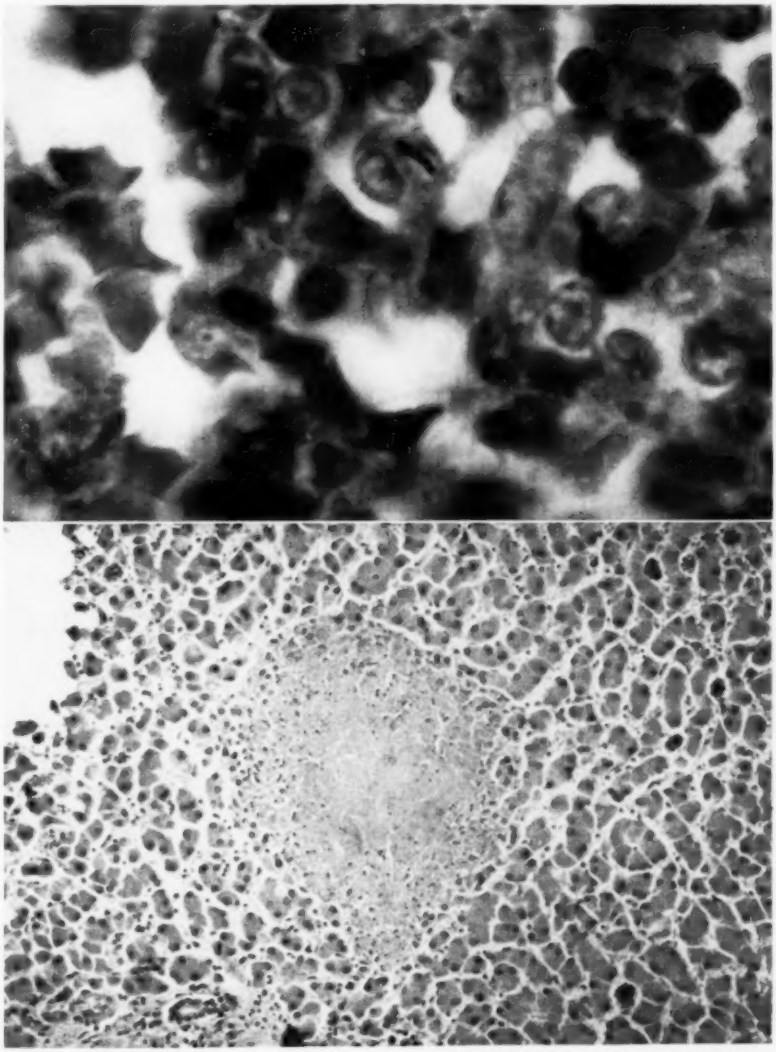

FIG. 2 (Upper). Tracheobronchial lymph node: Portion of lymph node structure free of caseonecrotic lesions showing reticuloendothelial cells with intracytoplasmic acid-fast bacilli. (Ziehl-Neelsen stain, × 1400)

FIG. 3 (Lower). Liver: Intralobular focus of miliary necrosis. Note absence of epithelioid and Langhans' giant cells. Within the sinusoids there are foci of leukemic lymphocytic cells. (Hematoxylin and eosin stain, × 210)

discovered at times at the periphery of the lesions. The small remaining lymph node parenchyma was made up of what appeared to be leukemic lymphocytic cells which had involved, on the other hand, the capsule of the nodes and extended into the neighboring adipose tissue. In this tissue, necrotic areas and some recent venous thrombi were also noted. Granulation tissue, epithelioid cells, Langhans' giant cells, or fibrosis were not present. The lesions were massively loaded with acid-fast bacilli; the number was even greater than that in the lungs.

In the leukemic lymph node parenchyma, numerous extra- and intracellular acid-fast bacilli were noticed; these bacilli were isolated or formed small clumps. The intracellular forms, which were in slight predominance, were located within the foamy cytoplasm of phagocytic reticuloendothelial cells. Some veins contained both extra- and intracellular acid-fast bacilli, isolated or forming small clumps; the intracellular forms were lodged in the cytoplasm of macrophagic elements (figure 2).

Liver: Miliary focal necrosis without cellular response was present throughout the liver. Bile pigment was frequently seen in the central liver cells and only at times in the Kupffer cells. The sinusoids were moderately engorged and exhibited occasional foci of leukemic lymphocytic cells which under low power looked like fetel extramedullary hematopoiesis. The portal spaces showed foci of necrosis surrounded very often by leukemic lymphocytic cells (figure 3). While some foci of necrosis were literally loaded with acid-fast bacilli, others were paucibacillary; in the latter the lowest count was 55 bacilli per field. From time to time, one or more bacilli were detected in the cytoplasm of liver, Kupffer, and leukemic cells. The necrotic foci displayed remnants of reticulum fibers which outlined the normal sinusoid pattern in the lobules.

Spleen: The white pulp especially revealed foci of necrosis as seen in the tracheobronchial and paratracheal lymph nodes. These foci were fairly rounded, of variable size, and sharply demarcated. Cellular response was absent. The splenic tissue between the lesions disclosed marked sinusoid engorgement and numerous leukemic lymphocytic cells in the reticular meshes (figure 4). All of the foci of necrosis were loaded with acid-fast bacilli, although the number was less in the smaller foci. In the splenic parenchyma between the lesions there were numerous phagocytic reticulo-endothelial cells containing intracytoplasmic acid-fast bacilli; these intracellular forms were frequently multiple and formed small clumps. Only a few extracellular acid-fast bacilli were found. Remnants of reticulum fibers were present in the necrotic foci which outlined the normal sinusoid pattern.

Kidneys: Marked recent hyperemia, capsular and cortical foci of leukemic lymphocytic cells, and lesions of cholemic nephrosis were noted in the kidneys.

Encephalon: A thorough study demonstrated moderate recent hyperemia, a good number of

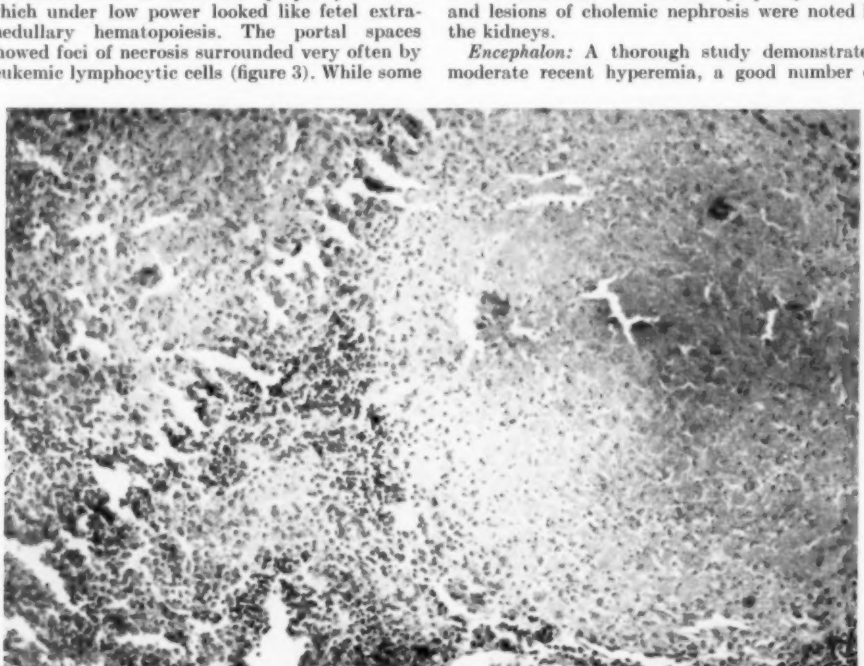

FIG. 4. Spleen: Caseonecrotic lesions fairly well demarcated from the splenic tissue. Cellular response is not present. (Hematoxylin and eosin stain, × 160)

erythrocytes in some His's spaces, areas of edema, and leukemic cells within some vessels of the leptomeninges.

Bone marrow: The marrow spaces were chiefly occupied by leukemic cells with the appearance of fairly mature lymphocytes. These cells are similar to those seen elsewhere. In the remaining scanty normal-looking marrow structure the megakaryocytes were apparently slightly increased in number. Scattered sharply demarcated foci of miliary necrosis were present and were surrounded by leukemic cells and marrow elements. Cellular response was absent. The search for acid-fast bacilli in the decalcified fragments was unfruitful. Sections taken from the sternum and body of the fourth lumbar vertebra were studied. Leukemic foci were also observed in the subepicardial fat tissue of the heart and in the adventitia of the thoracic aorta. Foci of miliary necrosis were also found in the pancreas and cortices of the adrenal glands; acid-fast bacilli were not investigated in these organs. The following staining methods were used: hematoxylin and eosin, Ziehl-Neelsen, Verhoeff's elastica, and Wilder's reticulum.

Post-mortem Bacteriology

Smears taken from the pulmonary lesions revealed myriads of acid-fast bacilli. Material taken from the same lesions was cultured in Sabouraud and Löwenstein-Jensen media, the results being negative. The cultures for fungi were held for six weeks, and those for tubercle bacilli, nine months.

Discussion

In this case, about five months after the discovery of a chronic lymphatic leukemia, a febrile process developed which lasted for about forty days until the death of the patient on August 20, 1957. The nature of the febrile process was identified only four days before death with the finding of acid-fast bacilli in the sputum. It is very likely that the tuberculous infection took place at the time when the patient developed a "cold" with progressive cough in the beginning of July, 1957.

The anatomic picture presented an evident recent primary pulmonary tuberculous infection with both intrabronchial and hematogenous dissemination. Terplan (27) has emphasized the need of post-mortem roentgenograms of the lungs with the major bronchi, trachea, larynx, and adjacent lymph nodes when anatomic studies on human tuberculosis are undertaken. Although it was not possible to get these films, careful dissection of the lungs did not disclose chalky, calcified, or ossified lesions or a distinct recent primary focus or primary infiltration. Moreover, the post-mortem roentgenograms of the larynx, trachea, major bronchi (cut at the hilus of each lung), and of all adjacent lymph nodes, which were obtained after fixation in 15 per cent formalin and in one piece, did not reveal lesions older than those seen grossly. Chalky, calcified, or ossified lesions were absent. Furthermore, the dissection of all of the bronchopulmonary, tracheobronchial, paratracheal, and venous angle lymph nodes failed to disclose lesions older than the caseonecrotic ones. Negative results were also obtained with the post-mortem film of the spleen, and the intestinal tract and mesenteric lymph nodes were free of older or recent tuberculous infection. O'Brien (9) in his review found that an old primary focus was reported nine times, and the same writer points out that some workers have stressed its absence. Siegmund (7) found no old primary focus in eight of the nine cases that he reported.

When the steroid therapy was begun, the tuberculous infection had continued for at least three weeks. It is not believed that the primary cause of the nonreactive histologic pattern of the lesions is related to that therapy. Corticotropin was given about three weeks after the clinical onset of the tuberculous process, the drug having been administered for three days and in a total amount of 120 mg. The opinion is that this dose of corticotropin is small and insufficient to produce the poor tissue response seen in clinical and experimental tuberculosis when the steroid therapy generally has been used over longer periods of time and in larger doses. Prednisone was given for five days, 20 mg. daily, four days after the withdrawal of corticotropin. If it be recalled that, in the case of Ruml and Haelig (26), in which a very poor cellular response was found, prednisone was given for twelve months, it may be seen that the amount of prednisone given in the present case was small and, therefore, insufficient to produce the same histologic picture as the case reported by Ruml and Haelig. It seems to the present writers that the two drugs (alone or together), given within an interval of four days and in a small amount to a tuberculous patient, cannot explain the nonreactive histologic picture in the present case. Cortisone was given during a five-day period about thirty-four days after the clinical onset of the tuberculous process, ten days after the withdrawal of corticotropin, and immediately after the withdrawal of prednisone. If there had been tissue response from the beginning of the febrile process, it could have been

seen in the histologic preparations in spite of the cortisone therapy because cortisone does not exert fibrolytic action and does not appear to have any demonstrable action on granulation tissue already formed (13). Moreover, the same thing could apply here that obtained in connection with corticotropin and prednisone. It cannot be denied, however, that the three drugs (alone or together) had an enhancing and worsening action in the present case before the administration of the antituberculous therapy, and it should be emphasized that, even with the antituberculous drugs, the body was unable to react. The process was nonreactive from the very beginning, and clinically corresponds to the type of chronic pyrexia of unknown origin which has been reported occasionally in cases of nonreactive tuberculosis (9). Furthermore, the present case falls within that group in which a pre-existing hematologic disease is present (9).

The tuberculin test has its importance. It has been reported that the adrenal hormones have as great a reducing or suppressing action on the cutaneous hypersensitivity in human beings as in experimental animals (28–34). Until 1954, according to O'Brien (9), the tuberculin skin test was practiced only three times in cases of nonreactive tuberculosis; in two cases, the reaction was weakly positive; and in the other one, negative. In the present case, the test gave a negative result. With this fact in mind, there are two interpretive possibilities. The first, that the patient was a true negative reactor; the second, that the patient was a true positive reactor, the absence of reaction being due to the cortisone, since this hormone was withdrawn two days before the test. Of course, the possibility of a true positive reaction if stronger concentrations of tuberculin had been used cannot be excluded; but, if the clinical elaboration is equivocal, the histologic findings seem to provide a definite interpretation. In tuberculosis, the actual known facts prove that necrosis is a phenomenon of hypersensitivity and is related to the bacillary proteins (12, 35). According to Canetti (35), the perifocal inflammation is most probably due to substances of inflammatory potential liberated in the tuberculous focus, these substances being bacillary proteins or products of cellular destruction of histaminic nature or of histamine-like activity. Canetti (35) gives more importance to the bacillary proteins which, as is known, provoke hypersensitivity reactions. In the present case, the marked perifocal inflammation in the lungs and the necrosis give histologic support to the possibility that the tissues of the patient were hypersensitive to the tuberculoproteins. The present writers believe that, if the finding of massive numbers of acid-fast bacilli is included, this case would have fulfilled the conditions under which (according to Rich and McCordock (8)) the nonreactive lesions are produced because they consider those lesions to be "merely the result of massive blood stream infection in the face of high allergy." On the other hand, as was said above, even with the help of the antituberculous therapy in the present case, there was no cellular response at all. The cultures taken before the specific treatment were positive for acid-fast bacilli within three weeks, and the cultures taken at the time of the autopsy and using the same medium were negative after nine months. From the cultural point of view, then, those acid-fast bacilli are probably nonviable. Although histologic evidence of bacillary destruction was not seen, it may be assumed that it took place anyway, because it seems to be the most probable mechanism to explain the lesions of necrosis and (at least in part) of perifocal inflammation.

Rich (12) has stated that in these cases there also is "at least a temporary depression of resistance that permits the free multiplication of the bacilli where they lodge." The findings in the present case also point to a low and ineffective resistance. The cellular debris in the necrotic foci and the presence of bacilli in the cytoplasm of the reticuloendothelial cells are interpreted as indications of a certain degree of resistance, although not effective. It seemed as if the antituberculous therapy, which was given during the last four days of the life of the patient, deeply interfered with the biology of the bacilli; but it did not find cooperation from the body to overcome the morbid process. It appeared as if the reticuloendothelial system would have a fairly well preserved phagocytic ability, but a markedly impaired lytic action. Whether or not this phagocytic-lytic dissociation could represent a defensive measure of the body against the hypersensitivity phenomena is not known. What apparently appears clear, however, is a notably reduced resistance.

Although it was not possible to identify the type of acid-fast bacilli, it may be assumed, because of the eugonic character of the cultures and the morphology of the organisms, that the

present organism belongs to the human variety of *Mycobacterium tuberculosis*.[2]

Summary

The case report is presented of a 70-year-old male who developed primary nonreactive tuberculosis in the course of a chronic lymphatic leukemia. The findings tend to support the concept that the nonreactive lesions are due to massive invasion of the blood stream by tubercle bacilli in persons with marked hypersensitivity and low and ineffective resistance.

Sumario

Tuberculosis Irreactiva

Preséntase la historia clínica de un sujeto de 70 años que manifestó tuberculosis irreactiva primaria durante la evolución de una leucemia linfática crónica. Los hallazgos tienden a apoyar el concepto de que las lesiones irreactivas proceden de la invasión masiva del torrente sanguíneo por bacilos tuberculosos en sujetos con destacada hipersensibilidad y poca e ineficaz resistencia.

Resume

Tuberculose non-réactive

Présentation de l'observation d'un cas où un malade âgé de 70 ans a présenté une tuberculose pulmonaire non-réactive au cours d'une leucémie lymphocytaire chronique. Les signes observés tendent à corroborer la théorie que les lésions non-réactives sont dues à l'invasion massive du courant sanguin par les bacilles tuberculeux chez des sujets possédant une hypersensibilité marquée et une résistance réduite et inefficace.

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Notes

GENETIC CONSIDERATIONS OF THE MECHANISMS INVOLVED IN PAS-RESISTANT TUBERCLE BACILLI

Para-aminosalicylic acid (PAS) is one of the most important antituberculous drugs; nevertheless, our knowledge of the genetic system involved in PAS-resistant tubercle bacilli is meager. Accordingly, it is the purpose of the present paper to deal with this problem from the genetic aspect and to correlate the findings with the general problem of variation of the organism.

Mycobacterium tuberculosis var. *hominis*, strain Aoyama-B, was used throughout the study. Ogawa's egg medium was used. Its composition and the method of inoculation were described in previous reports dealing with PAS resistance.[1, 2]

The survival ratio was calculated by inoculating a series of tenfold dilutions of cell suspension onto a set of tubes containing various concentrations of the drug. Considering the effect of PAS in retarding the growth of PAS-resistant mutants,[1] colony counts were made after six weeks of incubation at 37°C. If the growth was confluent, the colony count after four or five weeks of incubation was taken as the basis of calculation.

The population structure was expressed in terms of percentages of survivors of the total viable population on medium with PAS.

The generation time was measured according to Youmans and Youmans.[3] Five different dilutions of cell suspension were used for the measurement. The time of the first appearance of visible growth was plotted by an average of ten tubes.

The results are shown in table 1. The survival ratios of this strain of tubercle bacilli when exposed to PAS were discontinuous at two points. Discontinuance of the survival curve was observed at a point between 0.7 and 0.8 γ per ml., as well as between 2.0 and 5.0 γ per ml. The results indicate that susceptible cells are able to grow on medium with PAS concentrations of less than 0.7 γ per ml., and the survival curve slowly and continuously decreases until that concentration is reached.

The results shown in table 1 suggest that most mutants resistant to PAS concentrations of 0.8 γ per ml. are also resistant to 2.0 γ per ml. concentrations, and most mutants resistant to 5.0 γ per ml. are also resistant to 100.0 γ per ml.; therefore, there may be only two types of resistant mutants.

Survivors on medium with PAS concentrations above 0.8 γ per ml. are considered to be resistant mutants, for they are of rare occurrence and their properties of PAS resistance have been maintained throughout more than five transfers on drug-free medium made every six weeks.

Approximately one hundred colonies obtained by one-step selection of the parent strain by PAS concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 γ per ml. were designated as strains P1R, P2R, P5R, P10R, P20R, P50R, and P100R, respectively. The population structure of the strains was analyzed with respect to PAS resistance. The results are shown in table 2. The population structures of strains P1R and P2R were similar to each other, and those of strains P5R to P100R were also similar to each other; it was found that a significant difference existed between these two groups. The results also suggest that PAS resistance is determined by only two genotypes, and they agree

TABLE 1

SURVIVAL RATIOS OF M. TUBERCULOSIS VAR. HOMINIS (STRAIN AOYAMA-B) FOR VARIOUS PAS CONCENTRATIONS

PAS Concentration (γ per ml.)	Survival Ratio
0	1.00
0.01	1.02
0.02	0.96
0.05	0.80
0.1	0.49
0.2	0.48
0.5	0.30
0.6	0.19
0.7	0.033
0.8	2.16×10^{-5}
0.9	2.26×10^{-5}
1.0	2.81×10^{-5}
2.0	1.51×10^{-5}
5.0	1.20×10^{-8}
10.0	1.44×10^{-8}
20.0	1.70×10^{-8}
50.0	1.52×10^{-8}
100.0	1.22×10^{-8}

[1] TSUKAMURA, M.: Am. Rev. Tuberc., 1957, *75*, 608.

[2] TSUKAMURA, M.: Am. Rev. Tuberc., 1958, *77*, 346.

[3] YOUMANS, G. P., AND YOUMANS, A. S.: J. Bact., 1949, *58*, 247.

with the results obtained with respect to the survival curve.

If the genotype for higher degrees of PAS resistance, which is responsible for resistance to more than 5.0 γ per ml., was composed of mutations of a gene for lower degrees and any other gene, it would be expected from the survival ratios shown in table 1 that the mutation rate to mutation to the genotype of higher degrees might be approximately 10^{-3} in cells bearing the genotype of lower degrees. To test this possibility, the rate of appearance of mutants resistant to PAS concentrations of 100.0 γ per ml. was determined in the strain P2R obtained by one-step selection of the parent strain with 2.0 γ of PAS per ml. The results indicated that no mutants were found among approximately 4×10^7 viable cells of the strain, and such a possibility was discarded. It appears, therefore, that there is no relationship between these two genotypes for PAS resistance, and that mutation to the genotypes occurs independently.

Previously, the writer has shown that a marked heterogeneity exists in the population structure of PAS-resistant strains, and that the heterogeneity is derived from the nature of the strains themselves.[1, 2] Since it appeared probable that the heterogeneity was derived from variation of phenotypic expression in the existence of the same genotype, i.e., fluctuation, the following experiment was performed to test such a possibility. One clone was isolated from the PAS-resistant strains and analyzed for the population structure. As shown in table 3, heterogeneity was observed even among individual cells descended from a single cell. The results readily indicate that the heterogeneity is derived from the fluctuation of phenotype. Furthermore, it was remarkable that the growth rate of PAS-resistant mutants varied continuously, depending upon the PAS concentration. The results indicate that even mutants of lower degrees of resistance can grow on medium containing higher concentrations, but only at a delayed rate. They can represent a considerably abundant growth on such medium, however, if a very heavy inoculum is used. Therefore, the degree of PAS resistance should be determined by utilizing an inoculum of constant size that gives discrete colonies on drug-free medium.

TABLE 2

ANALYSIS OF THE POPULATION STRUCTURE OF VARIOUS ONE-STEP RESISTANT STRAINS

PAS	Percentage of Survivors*						
	P1R†	P2R	P5R	P10R	P20R	P50R	P100R
(γ per ml.)							
0	100	100	100	100	100	100	100
1	65	100	38	27	35	74	43
10	36	30	24	21	26	68	33
100	<0.06	<0.5	5	17	26	40	22
200	<0.06	<0.5	4	18	26	45	12
500	<0.06	<0.5	0.4‡	5‡	0.5‡	3‡	5‡

* Percentage of survivors on medium containing PAS of the total viable bacterial population. The number of survivors was determined by an average of ten replicates.

† Strains P1R, P2R, P5R, P10R, P20R, P50R, and P100R were obtained by one-step selection of the parent strain by 1, 2, 5, 10, 20, 50, and 100 γ of PAS per ml. of medium, respectively.

‡ Calculation was not accurate because the size of the colonies was too small.

TABLE 3

THE POPULATION STRUCTURE AND GENERATION TIME OF TWO STRAINS DESCENDED FROM A SINGLE CELL AMONG STRAINS P2R AND P100R-18P, RESPECTIVELY

Count Was Made After Four Weeks of Incubation

Strain*	PAS Concentration (γ per ml.)	Number of Survivors†	Significant Change by T-Test‡	Generation Time (hours)
P2R	0	51.0 ± 14.5	(−)	7.36
	0.1	40.8 ± 0.13	(−)	11.6
	1	31.5 ± 7.29	(+)	19.2
	2	30.9 ± 4.25	(+)	19.4
	10	0.0	(+)	38.4
P100R-18p	0	43.8 ± 13.6	(−)	7.25
	0.1	46.4 ± 12.9	(−)	7.92
	1	42.6 ± 8.60	(−)	7.62
	2	50.1 ± 8.22	(−)	9.10
	10	32.8 ± 4.08	(+)	9.38

* Strains obtained by one-step selection of the parent strain with 2 γ of PAS per ml. and obtained by eighteen-step selection of the parent strain with 100 γ of PAS per ml., respectively.

† Mean of ten replicates ± standard deviation.

‡ Significant difference at the 5 per cent level from the number of survivors on PAS-free medium.

In summary: It has been indicated that the PAS-resistant system of *Mycobacterium tuberculosis* var. *hominis*, strain Aoyama-B, consisted of two genotypes: one determining lower, and the other higher, degrees of resistance; and that these two genotypes are independent of each other.

Therefore, if expressed in genetic terms, PAS resistance appears to be determined by duplicate factors. It has also been indicated that a heterogeneous population structure characteristic of PAS-resistant strains is derived from fluctuation, i.e., variation of phenotypic expression within the same genotype.

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PRELIMINARY REPORT ON VACCINES PREPARED FROM GAMMA-IRRADIATED MYCOBACTERIUM TUBERCULOSIS AND BRUCELLA SUIS[1]

The direct action of high velocity ionizing radiations permits inactivation of pathogenic microorganisms with minimal structural, chemical, or antigenic denaturation. The dosage can be so calibrated as to permit retention of biologic properties, including metabolic activity, and thus presumably antigenic integrity.

Use of attenuated infectious agents to provoke active immunity is a well-established concept. Such vaccines include viable but relatively innocuous variants (BCG and abortus strain 19) and disease agents killed by heat or chemical procedures. The ideal vaccine would be nonproliferating and attenuated in a manner that would result in minimal alteration of the antigenic characteristics of the pathogen. Antigenic differences exist between virulent and avirulent strains as they do between living virulent organisms and those denatured by heat or chemicals. Sarber and associates[2] postulate a "virulence factor," a chemical complex associated with pathogenicity, essential to effective provocation of protective response. An example of the importance of antigenic structure is the early failure in this country to produce an effective immunization with the dissociated *Bord. pertussis*.

Dubos and his colleagues[3] have shown that killed virulent tubercle bacilli provoke immunity, and Sarber and co-workers[2] have produced effective immunization with UV irradiated organisms.

Although high energy ionizing radiations are effective in inactivating microorganisms, when microorganisms are irradiated in liquid suspensions much of the effect of these particles is due to indirect or secondary changes produced in the intracellular or surrounding fluids, particularly free-radical formation with consequent chemical denaturation of the bacterial substance.

Irradiation in the lyophilized state and at low temperatures reduces adverse effects on complex molecular structures, as well as reducing free-radical formation in intracellular fluids and consequent chemical damage. Pollard and associates[4] have done much to confirm Lea's[5] suggestion that in many cases a single primary ionization or a very small number of ionization events within the protein molecule can result in biologic inactivation of bacteria. The target diameter is the vital area of a bacterial cell. Calculated by Lea's method, it is approximately 57 to 63 mμ in size, or approximately 1/1,000 of the total cell volume. Numerous speculations have been made on the nature of the exact mechanism involved in inactivation of the bacterial cell by such relatively low dosages of ionizing radiation. The effect may be produced by disruption of a critical enzyme, by the direct action of ionization on the chromosomal nucleoprotein of the organism resulting in lethal mutation, or by induction, through the formation of radicals, of polymerization of unsaturated substances; the radical in this case acts simply as a catalytic agent stimulating normal spontaneously occurring processes. An inactivating dosage of radiation can be calculated to be approximately the amount that could affect one in 10^3 protein molecules.

Preliminary metabolic studies indicate that organisms subjected to inactivating dosages of irradiation can still respire and the antigenic integrity of the living infectious agent is retained, but the organisms are incapable of producing progressive disease, since they cannot multiply. They have the advantage of stability, ease of storage and transport, and are not subject to subtle mutations as are vaccines from living avirulent organisms. They do not, as do avirulent vaccines, differ antigenically from the disease-producing pathogens. The entire complex of antigenic structures present in the true pathogen is present, with minimal alteration.

In preliminary studies, vaccines have been prepared from *Mycobacterium tuberculosis* and *Brucella suis*. The vaccine is prepared from carefully selected pathogenic strains after frequent animal passage, ensuring retention of virulence. Logarithmic growth phase cultures are lyophilized and the sealed ampules are subjected to irradiation from a Co^{60} gamma source. The dose selected is the minimal inactivating dose plus a large safety factor. The lyophils thus treated can be stored and reconstituted at the point of use by the addition of water. Control aliquots are cultured to ensure their inability to reproduce.

[1] This study was supported by a research grant from the Los Angeles County Tuberculosis and Health Association, Los Angeles, California.

[2] Sarber, R. W., *et al.*: Am. Rev. Tuberc., 1950, *62*, 418.

[3] Dubos, R. J., *et al.*: J. Exper. Med., 1953, *97*, 221.

[4] Pollard, E. C., *et al.*: Prog. Biophys., 1955, *5*, 72.

[5] Lea, D. E.: Actions of Radiations on Living Cells, Cambridge University Press, London, 1946, p. 69.

TABLE 1

AGGLUTININ TITERS IN RABBITS IMMUNIZED WITH HEAT-KILLED AND GAMMA-IRRADIATED VACCINES*

Br. suis	Gamma-irradiated vaccine	1:5,120
	Heat killed vaccine	1:1,280
Br. abortus	Gamma-irradiated vaccine	1:2,560
	Heat-killed vaccine	1:1,280
Br. melitensis	Gamma-irradiated vaccine	1:1,280
	Heat-killed vaccine	1:1,280

* Administered subcutaneously at three-day intervals for one month.

Preliminary evaluation of the vaccine for tuberculosis was carried out in guinea pigs and Olitsky strain mice, and in rabbits and mice for the Brucella vaccine. The comparative agglutinin titers obtained in rabbits immunized with the gamma-irradiated vaccine and heat-killed vaccine are shown in table 1. A comparison between the protective effect of the irradiated vaccine as evinced by the survival time of mice infected with *Br. suis* after vaccination, and that afforded by Brucella killed by exposure to 60°C. temperatures for two hours with 0.05 per cent phenol added, is shown in table 2.

M. tuberculosis H37Rv was subjected to irradiation in a 100 curie Co^{60} source at a rate of 600 rep[6] per minute for a total dosage of 650,000 rep at ambient temperature and stored at −20°C. The vaccine was reconstituted by adding Sauton's medium to furnish a final suspension of 6×10^8 organisms per ml. No adjuvant was used.

Lyophilized BCG vaccine[7] was diluted in the same manner. The immunized animals were challenged with a strain of *M. tuberculosis* H37Rv[8] of known virulence, recently subjected to animal passage. Young male mice of the Olitsky strain were assigned at random to three groups.

Group I received no protective inoculum.

Group II received one intraperitoneal injection of 0.1 ml. of a 6×10^8 suspension of gamma-irradiated vaccine.

Group III received one intraperitoneal

TABLE 2

SURVIVAL TIMES OF MICE INFECTED WITH *Br. Suis* AFTER VACCINATION

Group	Total	Survivors							Survivors
		Days							
		2	4	6	8	10	12	14	
									per cent
Mice protected with gamma-irradiated vaccine	20	18/20	18/20	17/20	17/20	17/20	17/20	17/20	85
Mice protected with heat-killed vaccine	10	7/10	6/10	5/10	5/10	4/10	4/10	4/10	40

TABLE 3

TUKEY COMPARISON* EVALUATION OF DATA OF PROTECTIVE EFFECT OF BCG AND GAMMA-IRRADIATED M. TUBERCULOSIS IN MICE BASED ON LUNG DENSITY

Groups Compared (Mean Densities)		Confidence Limits of Difference in Means	Significance Level of Differences of Means
Unimmunized control $\bar{X} = 0.865$	BCG-immunized mice $\bar{X} = 0.839$	$+0.026 \pm 0.071$	Not significant at 0.05 level
Unimmunized control $\bar{X} = 0.865$	Gamma-irradiated vaccine-immunized mice $\bar{X} = 0.792$	$+0.073 \pm 0.071$	0.05
BCG-immunized mice $\bar{X} = 0.839$	Gamma-irradiated vaccine-immunized mice $\bar{X} = 0.792$	$+0.047 \pm 0.071$	Not significant at 0.05 level

* *See* DIXON, W. J., AND MASSEY, F. J.: Introduction to Statistical Analysis, ed. 2., McGraw-Hill, New York, 1957, pp. 153–155.

[6] Roentgen equivalent physical.

[7] Obtained through the courtesy of Dr. Sol Roy Rosenthal, Research Foundation, Chicago, Illinois.

[8] The writers are grateful to Dr. Anson Hoyt, University of Southern California and Barlow Sanatorium, Los Angeles, California, for his cooperation in these studies and for providing the strain of H37Rv and the Olitsky strain mice used.

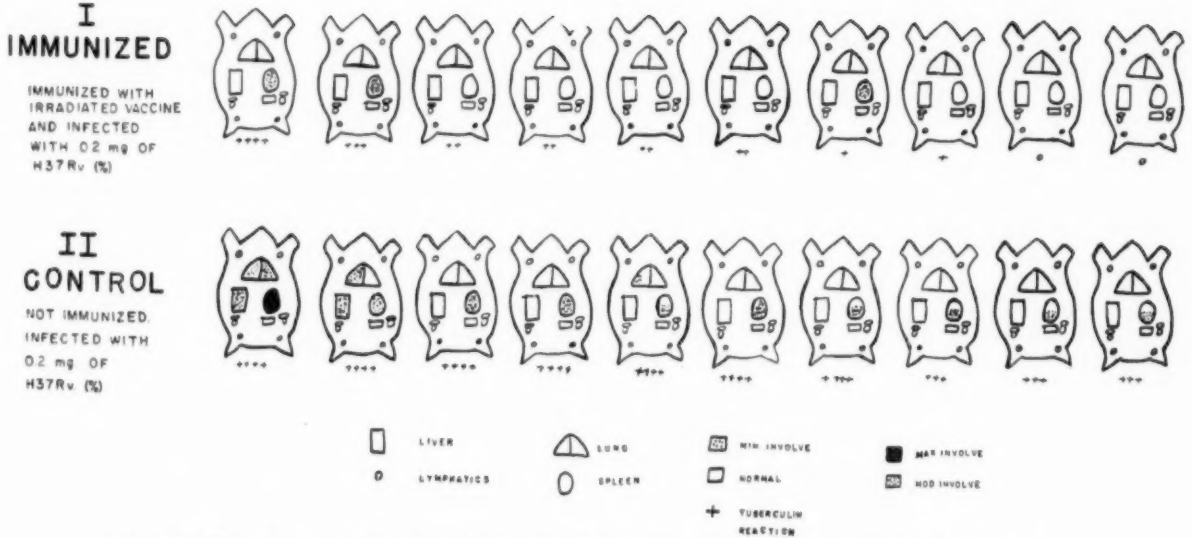

FIG. 1. Effect of gamma-irradiated *M. tuberculosis* vaccine in experimental tuberculosis in guinea pigs.

injection of 0.05 ml. of a 6×10^8 suspension of BCG.

All groups were challenged thirty-five days later by intravenous injection of 0.1 ml. of a suspension of 1.8×10^9 per ml. of *M. tuberculosis* in saline.

The animals were sacrificed fifty-seven days after challenge. Lung densities were determined by the procedure of Crowle.[9] The difference between the mean lung densities of the normal mice and the infected nonimmunized mice (0.701 and 0.865, respectively) was significant at the 1 per cent level. A comparison of the vaccinated groups by the Tuckey comparison test is shown in table 3.

The vaccine has also been tested in several series of guinea pigs. Vaccine prepared as above was reconstituted with water to contain 6×10^8 organisms per ml. Tuberculin-negative animals received 0.2 ml. of the suspension subcutaneously. Subsequently, they were infected by subcutaneous inoculation with 0.1 ml. of a suspension of H37Rv containing 0.1 mg. wet weight of the organisms. A control group of normal guinea pigs was similarly infected. The results of these experiments are shown in figure 1.

It was noted, as shown in figure 1, that 2 of the animals that exhibited increased resistance did not have positive tuberculin reactions following immunization, and that several others had only slight reactions. The fact that the gamma-irradiated vaccine provoked enhanced immunity without conversion of the tuberculin reaction is being studied. The vaccine produces no lesions.

In summary: A vaccine has been produced by inactivation by gamma-irradiation of *M. tuberculosis* that has proved to confer a protective effect against challenge infection comparable to that afforded by the standard viable attenuated vaccine (BCG). Preliminary results with a vaccine produced in the same manner from Brucella have been shown to be antigenic. Evaluation of this vaccine is in progress.

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[9] CROWLE, A. J.: Am. Rev. Tuberc., 1958, 77, 681.

[10] The writers wish to express their appreciation to Dr. W. S. Dixon of the Biostatistical Laboratory, Department of Preventive Medicine, U. C. L. A. Medical Center, for aid with the statistical calculations.

A PILOT STUDY OF THE TUBERCULIN TEST FOR CASE FINDING IN A GENERAL HOSPITAL

Saint Joseph's Hospital, South Bend, Indiana, has 175 beds for short-term illness with approximately 7,500 admissions per year. It is fully accredited and provides all of the services expected of a general hospital of medium size. Most of its patients are from St. Joseph County, which has a population of 225,000, a newly reported case rate of 29.3, and a mortality from tuberculosis of 4.9 per 100,000. These levels are somewhat less than state and national averages.

An active program for tuberculosis control has been conducted in this community for many years. Hospital beds are adequate for isolation and treatment. Tuberculin testing has been done in the schools for more than twenty-five years. About 30,000 persons have been examined each year by roentgenographic surveys since 1945. Other case-finding methods used include contact investigation and diagnostic and follow-up clinics. A case registry is maintained by the local health department.

An admission roentgenographic program has not been adopted previously by St. Joseph's Hospital because of space limitation, expense, and a lack of conviction of its value. More recently, the possible harmful genetic effect of radiation has been included with these objections.

In 1957 the medical staff agreed to try the tuberculin test as a device to screen admissions. It seemed to offer these possible advantages over a miniature roentgenographic program:

1. The danger and cost of roentgenography would be limited to those who had a definite indication for examination.
2. All tuberculous infection would be detected, including lesions not apparent in a standard film.
3. A file of positive reactors could be established for follow-up by roentgenographic surveys.
4. Insurance companies would pay for examinations done on individual order when they would not do so for a routine procedure.
5. Additional X-ray equipment and personnel would not be needed.

The hospital provided syringes, needles, and a nurse to do the tests. The Tuberculosis Association and Health Department supplied tuberculin, clerical help, and field nurses. Medical supervision was furnished by the present writer. The test was voluntary and given without charge.

The preparation used was PPD-S.[1] It was given intradermally according to the Mantoux technique. All admissions were offered the test with the following exceptions:

Those who had a negative tuberculin reaction within sixty days
Those who had a positive reaction within one year
Those who had a history of tuberculosis
Those whose attending physician objected because of the presence of dermatitis or other condition which he considered a contraindication
The newborn.

When a patient was admitted, a special form, prepared in duplicate, was attached to his chart. The test was given within twenty-four hours after entrance and interpreted about forty-eight hours later. If the reaction was positive, a red notice was placed upon the clinical record to remind his physician to order a chest film. The radiologist entered his opinion on the form when he read the film. The writer reviewed each case considered suspicious. Further studies were suggested to the attending physician when they were indicated. The original form was returned to the patient's chart, and the duplicate sent to the Health Department.

It soon became apparent that many patients left the hospital before a roentgenogram could be taken, or even ordered. Some left before the reaction had time to develop. Consequently, it was necessary for a field nurse to visit these early dischargees in their homes to inspect the injection site. A few who were ambulant reported to the clinic. The attending physician was notified about the result in each case. Some had standard films while others reported later for miniature films.

More than 3,800 people were admitted to St. Joseph's hospital between May 15 and November 15, 1957. About 2,700 received intradermal injections of tuberculin. Nearly 2,000 reactions were read in the hospital. Field nurses visited 454 persons who were discharged less than forty-eight hours after they entered. A total of 2,432 tests was completed. The number of tests completed was consistent for the first five months, ranging between 63 and 78 per cent. Completions dropped to 29 per cent during the last month.

The tuberculin reaction was not determined for a variety of reasons. One who is not familiar with hospitals of this sort may not realize how difficult it can be just to find patients. They move about surprisingly. Room accommodations are changed. Trips are made to X-ray laboratory, physiotherapy, delivery, and operating rooms. A few were not reported because they were not located

[1] Sharp and Dohme, 0.001 mg., 5 TU.

in time. Others were occupied in some manner which precluded administration of the test. This number increased sharply in the last month when the nurse at the hospital stopped the injections entirely, weeks before the termination date. More than 500 such cases are listed as "No report." A few were not given a test because they had had one previously. Only a small number refused. About 500 did not receive tuberculin for "other reasons," such as the presence of dermatitis, poor general condition, or simply because the attending physician did not think it was necessary. Approximately 1,100, nearly 30 per cent of the admissions, were not tested.

The tuberculin sensitivity of 52 patients had been determined before admission. These subjects were not re-tested, but they were included in the totals. Therefore, the status of the tuberculin reaction is known in 2,484, or 64 per cent of all admissions.

Reactions were reported in the hospital after forty-eight hours, somewhat later when they were seen following discharge. The induration of the skin was measured with a millimeter rule and graded according to size as described in *Diagnostic Standards* published by the National Tuberculosis Association. Twenty-six reactions were less than 5 mm. wide. These were classified as doubtful and grouped with negative. Twenty per cent had a weak response measuring 5 to 10 mm. (plus 1). Thirty-five per cent had induration 10 to 20 mm. in size (plus 2). In 30 per cent the reaction was larger than 20 mm. (plus 3). It was severe in 13 per cent, (plus 4). The percentage of positive reactions reported monthly varied from 22.7 to 26.1 per cent. The total number of positives was 603, an incidence of 24.3 per cent.

The level of tuberculous infection in the United States is usually estimated at about 33 per cent. The hospital patients in the present study appeared to have less than average, if the tuberculin test is a reliable gauge used in this manner. However, these people are not a representative cross-section of the population. As the community has a children's hospital, there were very few patients in the series who were less than fifteen years old. More important is the unequal use of hospital beds between males and females. Women outnumbered men six to one between the ages of fifteen and thirty-five, the child-bearing period. The disproportion continues, but to a smaller extent, up to forty-five (table 1).

Hospital patients are supposed to have more clinically significant tuberculosis than people in general, according to reports of admission chest roentgenographic programs. One might suspect that they would also have more tuberculous infection. However, the results of the tuberculin tests in the present study do not support this conclusion. To be sure, the two conditions are not necessarily parallel. Perhaps hospital patients do not control tuberculous infections well. As a group their resistance may be deficient. More infections may develop into clinical disease sufficient to be detected roentgenographically than is the case with healthy people.

The number who reacted to tuberculin increased with age, as expected. There were not enough subjects in this series less than fifteen years of age to establish an average for children. However, the school program shows a prevalence of 10 per cent

TABLE 1

TUBERCULIN SENSITIVITY BY AGE, SEX, AND RACE

Age	Males				Females				Total Male and Female White and Non-white	
	White		Non-white		White		Non-white			
	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors
0–4	5	0	0	0	7	0	1	0	13	0
5–14	20	0	2	0	22	0	3	0	47	0
15–24	53	9	4	0	314	8	27	11	398	8
25–34	92	28	6	33	566	16	46	41	710	19
35–44	112	37	5	40	269	23	23	35	409	27
45–54	127	49	7	57	162	37	13	69	309	44
55–64	122	41	6	66	137	32	9	33	274	57
65–74	101	26	9	67	96	36	6	33	212	30
75+	52	23	1	100	63	13	2	50	108	19
?	1		0	0	3		0		4	
Total.....	685	33	40	47	1,639	19	130	35	2,484	24

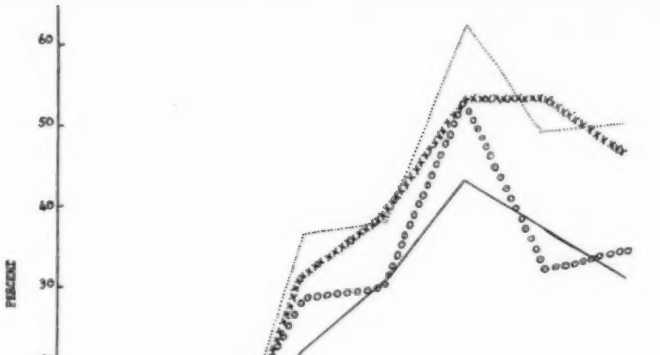

FIG. 1. Tuberculin sensitivity according to age, sex, and race.

during this period of life in this community. The level of infection in young, adult patients remains low, but increases sharply after twenty-five. A peak of 44 per cent was reached in the group between forty-five and fifty-four. After this the number of reactors decreased, especially in men (figure 1).

The reduction in tuberculin sensitivity in persons more than fifty appeared to be qualitative as well as quantitative. Weak responses (plus 1) increased from 19 per cent of all reactions in the younger groups to 25 per cent after fifty-four years. Perhaps the tubercle bacilli in the older lesions die. Then antibodies may decline and eventually disappear. No doubt the drop would be less marked if testing had included a follow-up injection of more concentrated tuberculin. On the other hand, it might be possible that lowered sensitivity might be nonspecific, associated in some obscure way with the process of aging.

Sex appeared to be a factor of importance. The difference was negligible in the young, but after twenty-five there were definitely more positive reactions in men. The ratio was at least 10 per cent greater for males in each decade until age sixty-four. After that the levels appeared to balance again.

Race also appeared to be responsible for variation in tuberculin sensitivity. The number of nonwhites in this study was too small to permit the establishment of accurate ratios by age. However, it seems obvious that there is more tuberculosis in non-white patients of all ages than there is in whites.

The percentage of positive reactions reported each month varied between 69 and 88 per cent, with 79 per cent as the average. One hundred and twenty-five subjects did not receive any roent-

TABLE 2

TIME AND TYPE OF CHEST ROENTGENOGRAMS

Roentgenograms before tuberculin test	17
Roentgenograms while in hospital	266
Roentgenograms after discharge	
Standard film	81
Survey film	114
Total roentgenograms	478

TABLE 3

COSTS OF TUBERCULIN TESTS

	Hospital	Tuberculosis Association	Health Department
Salaries	1,433.35	1,870.00	763.00
Supplies	106.28	423.43	
Transportation		334.96	100.00
Total	1,539.63	2,628.39	863.00

genographic examination. The status of the disease in these patients remains unknown. Chest films were obtained in almost 500 subjects. Twelve of these had clinically significant tuberculosis, an incidence of 0.5 per cent. Six of these lesions were minimal, four were moderately advanced. None of the lesions were active (table 2).

Apparently there was less clinical tuberculosis in this group of patients than has been reported from routine roentgenographic surveys of hospital admissions, which usually list a rate of 1 to 3 per cent. The fact that no active cases were found is probably not significant since the total number of persons examined was small for a case-finding program. Perhaps the favorable comparison is due to the effectiveness of the measures to control this disease which have been in use for many years. Vital statistics indicate that this may be so because the new case and mortality rate in this community are below state and national levels. The incidence of tuberculous infection would have been greater if the test had been repeated using stronger tuberculin, but then the results might have been misleading due to the inclusion of false positives.

The cost of the study was slightly more than $5,000. Personnel service accounted for about 80 per cent of the expense. The remainder was about equally divided between purchase of supplies and transportation for nurses who followed discharged patients into their homes to complete the tests (table 3). This task placed quite a burden upon the personnel of the Association and Health Department. The cost of the roentgenograms is not included because they were paid for individually, or the examination was made during a survey. The net cost per tuberculin test was $2.07.

Several conclusions are possible from this study. The tuberculin test is not a practicable way to prevent the admission of unrecognized tuberculosis into a general hospital. Many patients are discharged before reactions have time to develop or before a chest film can be obtained. The procedure should be more useful for admissions to institutions for the care of chronic disease, where the length of stay is greater.

The combination of a tuberculin test and a chest roentgenogram, as it was used in this study, provides a much more accurate survey of any group of people for tuberculosis than can be obtained by the use of roentgenography alone. Most of these patients reacted negatively to tuberculin. They can be assured they do not have tuberculosis without further expense or unnecessary and undesirable exposure to radiation. All of the reactors know that they contain a potential threat to their health, including many whose infections would not be discovered by roentgenography.

In summary: It is concluded that the combined use of tuberculin and roentgenography should be a part of every complete individual examination, whether it is made in the hospital or in a doctor's office. The physician will have the satisfaction of knowing that his diagnosis is thorough and that he is practicing good preventive medicine, either of which reflects to the benefit of the patient.

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August 21, 1958

FAILURE OF POLYOXYETHELENE ETHER (TRITON WR 1339) TO PROTECT AGAINST TUBERCULIN SHOCK IN GUINEA PIGS

Polyoxyethelene ether (Triton WR 1339®), while not bacteriostatic for virulent tubercle bacilli *in vitro*, has the ability to suppress tuberculosis in experimental animals.[1-3] In the course of studies in this laboratory, it was found that the administration of polyoxyethelene ether to tuberculin-sensitive guinea pigs two hours before the intradermal injection of PPD slightly depressed their skin sensitivity to tuberculin and essentially obliterated the lytic effect of tuberculin on their circulating leukocytes.[4] Harasawa and associates[5] have reported that mononuclear cells from tuberculin-sensitive guinea pigs treated with polyoxyethelene ether failed to transfer tuberculin sensitivity to normal guinea pigs.

In view of these findings it seemed of interest to determine whether the administration of polyoxyethelene ether to tuberculin-sensitive guinea pigs modifies in any way the course of tuberculin shock.

Male albino guinea pigs were made hypersensitive to tuberculin by a single intramuscular injection of 5 mg. of heat-killed tubercle bacilli, H37Rv,[6] in 1 ml. of sterile paraffin oil. Seven weeks later the animals were used in the tuberculin shock tests. A 12.5 per cent solution of polyoxyethelene ether[7] was prepared in 0.9 per cent sodium chloride, sterilized in the autoclave, and given to the treated animals in a dose of 300 mg. per kg. of body weight. The control animals received an equal volume of saline. Injections were made subcutaneously in the groin. Tuberculin shock was produced by the intraperitoneal injection of 1 ml. of undiluted OT.[8] This lot of OT, in the dosage described, was nontoxic for normal, nonsensitized guinea pigs.

Two separate experiments were done. In the first, 17 sensitized guinea pigs were used. Nine animals received polyoxyethelene ether and 8 animals received saline two hours prior to the injection of OT. In the second study, 6 experimental and 6 control guinea pigs were administered polyoxyethelene ether or saline, respectively, twenty-four hours prior to injection of OT.

TABLE 1

EFFECT OF POLYOXYETHELENE ETHER (TRITON WR 1339) ON TUBERCULIN SHOCK IN GUINEA PIGS

Group	Experiment Number 1*			Experiment Number 2†		
	Number of Guinea Pigs	Number of Survivors		Number of Guinea Pigs	Number of Survivors	
		(24 hours)	(48 hours)		(24 hours)	(48 hours)
Saline	8	2	2	6	4	3
Polyoxyethelene ether	9	2	1	6	3	3

* Saline or polyoxyethelene ether administered two hours prior to injection with OT.

† Saline or polyoxyethelene ether administered twenty-four hours prior to injection with OT.

The results are summarized in table 1. It will be seen that the administration of polyoxyethelene ether to tuberculin-sensitive guinea pigs, either two hours or twenty-four hours prior to injection with OT, failed to protect the animals from death by tuberculin shock. Within three hours after the injection of OT, all of the animals, regardless of whether they had received polyoxyethelene ether or saline, showed signs of shock, with ruffled hair, prostration, and increased respiration. Autopsies performed on animals that died revealed large amounts of fluid in the peritoneal and thoracic cavities. The spleens, livers, and intestines were hemorrhagic.

The choice of time intervals between the injection of polyoxyethelene ether and OT was based on the results of previous studies in this laboratory.[4] The administration of 300 mg. of polyoxyethelene ether per kg. to tuberculin-sensitive guinea pigs two hours before the intradermal injection of PPD depressed their skin sensitivity to tuberculin. Also, it was found that, with the dosage level used in the present study, maximal plasma levels of polyoxyethelene ether occurred approximately twenty-four hours after injection. Since it might have some bearing on the present findings, it should perhaps be pointed out that the depress-

[1] CORNFORTH, J. W., *et al.*: Nature, 1951, *168*, 150.

[2] SOLOTOROVSKY, M., AND GREGORY, F. J.: Am. Rev. Tuberc., 1952, *65*, 718.

[3] KATO, L., AND GOZSY, B.: Am. Rev. Tuberc., 1957, *75*, 684.

[4] PATNODE, R. A., AND HUDGINS, P. C.: J. Exper. Med., 1958, *107*, 55.

[5] HARASAWA, M., *et al.*: Kekkaku, 1957, *32*, 644.

[6] Original culture kindly supplied by Dr. William Steenken, Jr., Saranac Lake, New York.

[7] Obtained from Winthrop Laboratories, New York.

[8] Generously supplied by Dr. R. Y. Gottshall, Michigan Department of Health, Lansing, Michigan.

ing effect of polyoxyethelene ether on tuberculin skin sensitivity was not particularly striking (12 per cent reduction in mean diameter of induration at forty-eight hours). The fact that polyoxyethelene ether protects circulating leukocytes against lysis by PPD,[4] but affords no protection against tuberculin shock, suggests that *in vivo* leukocytolysis is not an essential feature of the systemic reaction to tuberculin.

In summary: The administration of polyoxyethelene ether (Triton WR 1339) (300 mg. per kg.) to tuberculin-sensitive guinea pigs, either two hours or twenty-four hours prior to injection with OT, failed to protect the animals from death by tuberculin shock.

ROBERT A. PATNODE
PAUL C. HUDGINS
Tuberculosis Research Laboratory, Veterans Administration Hospital
Washington, D. C.

November 10, 1958

Books

THE PASTEUR FERMENTATION CENTENNIAL 1857–1957. A Scientific Symposium on the Occasion of the One Hundredth Anniversary of the Publication of Louis Pasteur's *Mémoire sur la fermentation appelée lactique*. Pp. 207, Chas. Pfizer & Co., Inc. New York, 1958.

In 1857 Louis Pasteur published his *Mémoire sur la fermentation appelée lactique*, a short paper destined to open a new road in understanding the hitherto puzzling phenomenon of fermentation and a whole train of morbid processes in plants, animals, and human beings as well. In this country the one hundredth anniversary of the epoch-marking contribution was celebrated in a scientific symposium arranged by Chas. Pfizer and Co., Inc., at which some twenty distinguished biologists presented papers on the significance of Pasteur's discoveries and the directions modern studies are taking in clarifying the pathogenesis of infectious disease and aiding in the laboratory and industrial development of antimicrobial substances for its control. Pasteur's grandson, L. Pasteur Vallery-Radot, recalled his own memories of his celebrated forebear; Pasteur's devoted biographer, René J. Dubos, re-analyzed Pasteur's still growing place in the history of science, and the Ambassador of France to the United States, his Excellency Hervé Alphand, expressed his faith that Pasteur's influence will continue to bind the nations of the world together in peaceful, constructive effort. The technical papers presented in the symposium by American, French, British, Russian, Japanese, and Cuban representatives, not only furnish information of immediate worth, but point one way in which this fervent hope may be fulfilled.

ESMOND R. LONG
Pedlar Mills, Virginia

PRACTICAL ALLERGY. By M. Coleman Harris and Norman Shure. Pp. 471, F. A. Davis, Philadelphia, Pennsylvania, 1957.

The authors state in their preface that their text is intended as a comparatively simple one which can be used by the physician not specifically trained in the field of allergy, who wishes to carry on the management of allergic patients. It is also offered to the medical student as a useful, uncomplicated, and practical text. In general, the authors have accomplished their purpose admirably. The book is well printed and well organized into twenty-two chapters, which cover both the theoretical and practical aspects of allergy.

In place of specific references, a general bibliography for suggested supplemental reading is appended. The authors state that no specific references should be necessary since the text contains a concensus of the most acceptable current information on the subject. However, there are an unfortunate number of exceptions throughout the book.

For example, on page 151, the authors, in discussing the mechanism of the development of cardiac asthma, make the surprising statement that "it is thought to be precipitated or triggered by some event that occurs while the patient is asleep, such as slipping in bed, nightmare, cough, or excessive warmth of the body." It is doubted that the cardiologists would agree with this explanation.

The reviewer has not had the authors' experience as reported on page 153 to the effect that dilated bronchi are usually visible on roentgenograms of patients with bronchiectasis. Such a statement may give the physicians not specializing in this field a false sense of security from a negative roentgenographic report for patients who should have bronchography.

In the section on thrombocytopenic purpura on page 196, the authors have not been sufficiently clear in discussing the immune mechanism when they state that a platelet agglutinating factor has been found in the blood of persons which, transfused into nonallergic persons, has produced a marked falling in platelets. They fail to state that the recipient of this blood must first take the incriminated drug before thrombocytopenia occurs.

On page 288, the statement is made that the lupus erythematosus factor acts as a lysin to leukocytes. The lupus erythematosus factor has been shown to act on nuclei of leukocytes, depolymerizing the desoxyribonucleic acid, prior to phagocytosis of the depolymerized nuclei of the other leukocytes to develop the lupus erythematosus cell.

On page 294, the statement is made that the use of penicillin and sulfa drugs in the treatment of the sore throat has proved successful in stopping the progress of the rheumatic fever syndrome. This statement is somewhat vague in that it has not been clearly stated that it is penicillin but not sulfa drugs which are effective in this situation. Sulfa drugs are effective only as a prophylactic measure.

The sections devoted to practical allergy are far more authoritative and less likely to raise

differences of opinion among allergists. Two points deserve mention. On page 116, the authors advise disregarding mild (skin test) reactions to house dust antigen and considering as important only the marked positive reactions. They state that in this manner misinterpreting a primary irritant reaction may be avoided. It may have been worth while at this point to stress that the history is as important as the skin test and that a mildly positive reaction in the presence of a clear-cut history of aggravation of symptoms with exposure to dust merits respect and immunization with dust extract. That this would be a fair statement of the authors' opinion is reflected by their comment in discussing cattle hair: that no piece of furniture or room should be ripped apart unless the clinical symptoms coincide with the allergy tests for the animal hair involved.

The second point is raised by the authors' statement on page 30 that the areas along the coasts of the United States are *relatively* free of alternaria, hormodendrum, and Aspergillus (italics ours). While this statement is true as it appears, it would have been in the interest of clarity to point out that these molds cause definite symptoms even though they are in much lower concentrations in coastal regions.

These criticisms are not meant to imply that this book is other than a first rate job. The authors have succeeded admirably in their difficult task of covering a great deal of material in a relatively short book for physicians who have not been specifically trained in the field of allergy. For such physicians, this book is recommended as an intensely practical, readable volume.

MURRAY DWORETZKY
New York, New York

CEREBROSPINAL FLUID PRODUCTION, CIRCULATION, AND ABSORPTION. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecelia M. O'Connor. Pp. 355, Little, Brown and Company, Boston, Massachusetts, 1958. Price, $9.00.

This volume contains the formal presentations and informal discussions of the symposium on "Production, Circulation and Absorption of the Cerebrospinal Fluid," held in London in May, 1957. The papers, fifteen in number, concern the embryology and anatomy of the choroid plexus and its behavior in tissue culture, the neural structure of the meninges and choroid plexus, the production and circulation of the cerebrospinal fluid, the effect of vitamins upon the cerebrospinal fluid, the relationship between the cerebrospinal fluid and the central nervous system, neuropathologic aspects of the cerebrospinal fluid pathways, the mechanism of hydrocephalus, and the problems of experimental spinal anesthesia.

The conference emphasized basic aspects of the problems, only five of the fifteen papers being prepared by clinicians. The authors, most of whom are British, maintain a high standard, and most of the presentations are succinct, relevant to the subject, and several are deserving of special mention: Kappers' study on the telencephalic choroid plexus during human ontogenesis, Wislocki and Ladman's electron microscopic studies of the fine structure of the mammalian choroid plexus, Lumsden's observations on the choroid plexus in tissue culture, and Selverstone's masterful reinterpretation of the inferences to be drawn from radioisotope studies of the formation and absorption of the cerebrospinal fluid.

The discussions of the individual papers are illuminating and are presented in detail. Each of the discussors extends to the utmost limits the inferences to be gained from the demonstrated findings, considering what new avenues of approach might be employed to extend each particular facet of knowledge or reinterpreting the inferences drawn from the findings. All in all, these are unusually high-level and purposeful discussions.

Finally, while emphasis is on data, perhaps the greatest success of the volume is in pointing up how little is fully known and how much is unknown. The gaps in knowledge concerning production and absorption of the cerebrospinal fluid, regulation of cerebrospinal fluid pressure, and the circulation of the fluid are constantly emphasized.

The book will be useful to all anatomists, physiologists, neurologists, and neurosurgeons. It is clearly printed, the illustrations are of excellent quality, and it is well indexed.

CHARLES E. WELLS
New York, New York

BOOKS RECEIVED

THE CARE OF THE GERIATRIC PATIENT. Edited by E. V. Cowdry. Pp. 438, The C. V. Mosby Company, St. Louis, 1958. Price, $8.00.

LA MALADIE RHUMATISMALE (SYNDROME DE BOUILLAUD ET SÉQUELLES CHRONIQUES) TUBERCULOSE CARDIO-ARTICULAIRE POLYSÉRITE TUBERCULEUSE. By François Dalencour. Pp. 283, Doin & Cie, Paris, 1957.

LA BACILLÉMIE TUBERCULEUSE ET LA PHTISIOGÉNÈSE. By François Dalencour. Pp. 298, Doin & Cie, Paris, 1954.

TUBERCULOSIS: PREVENTION AND CONTROL. By

H. W. Hetherington and Fannie W. Eshleman. Pp. 404, G. P. Putnam's Sons, New York, 1958. Price, $6.50.

LE CATHÉTÉRISME DU COEUR DROIT ET DES ARTÈRES PULMONAIRES EN PNEUMOLOGIE. By Pierre Trocmé and Jacques Chedal. Pp. 110, Masson et Cie, Editeurs, Paris, 1958. Price, 1,600 fr.

STAPHYLOCOCCAL INFECTIONS. By Ian Maclean Smith. Pp. 180, The Year Book Publishers, Inc., Chicago, 1958. Price, $4.25.

MONOGRAPHS ON NEOPLASTIC DISEASE AT VARIOUS SITES. VOLUME I: CARCINOMA OF THE LUNG. Edited by J. R. Bignall. Pp. 298, The Williams and Wilkins Co., Baltimore, 1958. Price, $10.50.

ANTIBIOTICS MONOGRAPHS: No. 8. CHLOROMYCETIN (CHLORAMPHENICOL). By Theodore E. Woodward and Charles L. Wisseman, Jr. Pp. 159, Medical Encyclopedia, Inc., New York, 1958. Price, $4.00.

ANTIBIOTICS MONOGRAPHS: No. 9. PENICILLIN. By Harold L. Hirsh and Lawrence E. Putnam. Pp. 148, Medical Encyclopedia, Inc., New York, 1958. Price, $4.00.

ANTIBIOTICS MONOGRAPHS: No. 10. STREPTOMYCIN AND DIHYDROSTREPTOMYCIN. By Louis Weinstein and N. Joel Ehrenkranz. Pp. 116, Medical Encyclopedia, Inc., New York, 1958. Price, $4.00.

TUMORS OF THE LUNGS AND MEDIASTINUM. By B. M. Fried. Pp. 467, Lea and Febiger, Philadelphia, 1958. Price, $13.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

PRELIMINARY PROGRAM, MEDICAL SESSIONS, AMERICAN TRUDEAU SOCIETY ANNUAL MEETING

MONDAY, MAY 25

10:05 a.m.–12:15 p.m.

1A Clinical Tuberculosis

Patterns of Disease Associated with Atypical Mycobacteria in Texas—DANIEL E. JENKINS, M.D., DAVID BAHAR, M.D., IRVING CHOFNAS, M.D., RILEY FOSTER, M.D., FRANCES WHITCOMB, M.S., MARTHA FOSTER, M.S., ELIZABETH JONES, B.A., AND HELEN MCGEE, B.S., Houston, Texas

Chronic Pulmonary Tuberculosis in Individuals with Known Previous Primary Tuberculosis—EDITH M. LINCOLN, M.D., LILIAN A. GILBERT, M.D., AND SOLEDAD M. MORALES, M.D., New York, N. Y.

Corticosteroids in the Treatment of Pulmonary Tuberculosis—G. H. HAMES, M.D., Lantana, Fla.

The Impact of Corticosteroid Hormones in the Treatment of Tuberculous Meningitis—B. F. VOIJAVEC, M.D., AND R. F. CORPE, M.D., Rome, Ga.

Relapse Experience of Optimally Treated Patients—THOMAS F. SHEEHY, JR., M.D., Seattle, Wash.

Chemotherapy in the Surgical Treatment of "Salvage Cases" of Pulmonary Tuberculosis—MARJORIE M. PYLE, M.D., HIRAM T. LANGSTON, M.D., AND KARL H. PFUETZE, M.D., Chicago, Ill.

The Treatment of Tuberculosis with Alternating Antituberculous Drugs—E. T. PEER, M.D., Ontario, Canada

Discussion

10:05 a.m.–12:15 p.m.

1B Pulmonary Physiology

The Influence of Increased Intra-alveolar Pressure on the Uptake of Carbon Monoxide by the Lungs and on the Diffusing Capacity of the Pulmonary Membrane and Pulmonary Capillary Volume in Man—THEODORE C. SMITH, M.D., JOHN H. RAMLO, M.S., D.D.S., RICHARD A. COOPER, B.S., CARL B. WESTON, B.S., AND JOHN RANKIN, M.D., Madison, Wisc.

A Closed Circuit Method of Determining Carbon Monoxide Diffusing Capacity of Human Lung—H. G. BOREN, M.D., AND R. E. COLLINS, PH.D., Houston, Texas

The Simultaneous Relationships of Diffusing Capacity and Pulmonary Capillary Blood Flow to Alveolar Volume from Areas of the Lung Differing in Ventilation: In Normals and Patients with Emphysema—WILLIAM S. SPICER, JR., M.D., Baltimore, Md., ROBERT L. JOHNSON, JR., M.D., Dallas, Tex., AND ROBERT E. FORSTER, M.D., Philadelphia, Pa.

Dimensional Responses of Bronchi to Airway Pressure, Gases, and Drugs—KAYE H. KILBURN, CAPTAIN, MC, Denver, Col.

Cardiopulmonary Function in Chronic Obstructive Emphysema—M. HENRY WILLIAMS, JR., M.D., AND LENORE R. ZOHMAN, M.D., Valhalla, N. Y.

The Syndrome of Pulmonary "Hyperinflation": An Early Stage of Chronic Pulmonary Insufficiency: A Preliminary Report of 40 Cases—JAMES C. SYNER, MAJOR, MC, AND JOEL H. RICHERT, MAJOR, MC, Denver, Col.

The Roentgenographic Determination of the Total Lung Capacity in Health, Emphysema, and Congestive Heart Failure—JOHN A. PIERCE, M.D., HOWARD J. BARNHARD, M.D., JOHN JOYCE, AND JOSEPH BATES, M.D., Little Rock, Ark.

The Estimation of Ventilatory Function by Means of Fluoroscopy—GORDON L. SNIDER, M.D., AND ALLAN SHAW, Chicago, Ill.

Pulmonary Vascular Resistance in Acquired Heart Disease—BERNARD L. CHARMS, M.D., AND BERNARD L. BROFMAN, M.D., Cleveland, Ohio

12:30 p.m.–1:45 p.m.

Sandwich Seminar I*

Solitary Pulmonary Nodules—JOHN D. STEELE, M.D., San Fernando, Calif., *Moderator*

Panelists: BENJAMIN BURROWS, M.D., Chicago, Ill., and HENRY GARLAND, M.D., San Francisco, Calif.

Sandwich Seminar II*

Practical Application of Pulmonary Function Testing—WARD FOWLER, M.D., Rochester, Minn., *Moderator*

Panelists: GILES F. FILLEY, M.D., Denver, Col., and EDWARD A. GAENSLER, M.D., Boston, Mass.

Sandwich Seminar III*

Management of Re-treatment Problems in Tuberculosis—WILLIAM B. TUCKER, M.D., Washington, D.C., *Moderator*

Panelists: JOHN H. MCCLEMENT, M.D., New York, N. Y., SIDNEY DRESSLER, M.D., Denver, Col., ROBERT R. SHAW, M.D., Dallas, Texas, and NICHOLAS D'ESOPO, M.D., West Haven, Conn.

2:00 p.m.–3:30 p.m.

2A Symposium on Pulmonary Emphysema

RICHARD V. EBERT, M.D., Little Rock, Ark., *Moderator*

The Elastic Fiber System of the Human Lung—CLAYTON G. LOOSLI, M.D., Los Angeles, Calif.

Pathology—DAVID M. SPAIN, M.D., Brooklyn, N. Y.

Relationship Between Bronchitis and Emphysema—CHARLES M. FLETCHER, M.D., London, England

Pathology and Pathogenesis—KENNETH MCLEAN, M.D., Rochester, Minn.

Localization of Pulmonary Emphysema—DAVID V. BATES, M.D., Montreal, Canada

Basic Physiological Abnormality—GILES F. FILLEY, M.D., Denver, Col.

Discussion

2:00 p.m.–3:30 p.m.

2B Differentiation of Mycobacteria

Atypical Acid-fast Bacilli—SHIRLEY MCMILLEN, M.A., AND DANIEL S. KUSHNER, M.D., Chicago, Ill.

An Attempt to Sub-group the Acid-fast Bacilli on the Basis of Their Catalase Activities: Preliminary Report—G. P. KUBICA, PH.D., AND GLEASON POOL, B.S., Chamblee, Ga.

A New, Rapid Concentration and Differential Culture Technique for the Mycobacterium and Nocardia Organisms—JAMES B. GRAY, B.S., EDITH REINISH, M.S., AND SANFORD CHODOSH, M.D., Boston, Mass.

Preliminary Studies on the Identification of Atypical Nocardia Species Through Application of Bacteriophage—HELEN J. SHADOMY, M.A., AND O. A. PLUNKETT, PH.D., Los Angeles, Calif.

The Intracutaneous Inoculation of Guinea Pigs as a Means of Determining the Virulence of Drug-resistant and Anonymous (Atypical) Acid-fast Bacilli: Preliminary Report—G. P. KUBICA, PH.D., R. EDWARD BEAM, B.S., ANNIE L. VESTAL, B.S., AND GLEASON POOL, B.S., Chamblee, Ga.

A Comparative Study of M. Avium and the "Battery" Type of Atypical Acid-fast Bacillus—SEYMOUR FROMAN, PH.D., LOIS SCAMMON, B.SC., EMIL BOGEN, M.D., AND J. P. MYLES BLACK, M.D., Olive View, Calif.

Observations on Nonculturable Acid-fast Bacilli in Resected Pulmonary Lesions—LAWRENCE G. WAYNE, PH.D., San Fernando, Calif.

Nonacid-fast or Chromophobic Microorganisms in the Tuberculous Lesions of the Human Lung—WALENTY NYKA, M.D., Baltimore, Md.

Discussion

3:40 p.m.—5:00 p.m.

2C Tuberculosis Epidemiology

Finding Tuberculosis through Family Contact Investigation at a Children's Tuberculosis Clinic—KATHERINE H. K. HSU, M.D., Houston, Texas

Screening Procedures for Pulmonary Disease in Pregnant Patients—DOROTHEA D. GLASS, M.D., FRUMA W. GINSBURGH, AND KATHARINE R. BOUCOT, M.D., Philadelphia, Pa.

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* Each seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Trudeau Society.

Environmental versus Constitutional Factors in the Development of Tuberculosis among Negroes—JULIUS KATZ, M.D., AND SOLOMON KUNOFSKY, Albany, N. Y.

Tuberculosis Control in Northern Laborador—W. A. PADDON, M.D., North West River, Labrador

Legal Custody of Recalcitrant Tuberculosis Patients: Report on a Hospital Program—ROBERT H. BROWNING, M.D., AND IRVING PINE, M.D., Columbus, Ohio

Discussion

4:15 p.m.–5:00 p.m.

Lecture

The Anatomical and Physiological Alteration of the Lung due to Heart Disease—HOWARD B. BURCHELL, M.D., Rochester, Minn.

Introduction by HANS H. HECHT, M.D., Salt Lake City, Utah.

4:15 p.m.–5:30 p.m.

Demonstration I

The Gel-Diffusion Precipitation Diagnostic Test for Tuberculosis—ROBERT C. PARLETT, M.D., AND GUY P. YOUMANS, M.D., Chicago, Ill. Limited to 150 physicians. Reservations must be obtained in advance from the American Trudeau Society.

4:15 p.m.–5:30 p.m.

Demonstration II

Devices for Artificial Respiration and Tests of Pulmonary Function—GEORGE SAXTON, M.D., AND JANET WOLTER, M.D., Chicago, Ill. Limited to 25 physicians. Reservations must be obtained in advance from the American Trudeau Society.

TUESDAY, MAY 26

9:00 a.m.–10:45 a.m.

3A Nontuberculous Pulmonary Disease

The Treatment of Eight Cases of Deep Mycotic Infection with Amphotericin B, with Particular Emphasis on Drug Toxicity—HALLARD W. BEARD, Captain, MC, JOEL H. RICHERT, Major, MC, AND RICHARD R. TAYLOR, Lt. Colonel, MC, Denver, Colo.

Aspergillosis—SYDNEY M. FINEGOLD, M.D., DRAKE WILL, M.D., AND JOHN F. MURRAY, M.D., Los Angeles, Calif.

Clinical and Physiological Results Following Radioactive Iodine (I^{131}) in the Treatment of Chronic Pulmonary Insufficiency—LOIS T. ELLISON, M.D., ROBERT G. ELLISON, M.D., AND WILLIAM F. HAMILTON, PH.D., Augusta, Ga.

The Use of Intermittent Positive Pressure Breathing to Prevent Carbon Dioxide Retention Induced by Oxygen in Pulmonary Emphysema—RICHARD T. CATHCART, M.D., AND WILLIAM FRAIMOW, M.D., Philadelphia, Pa.

The Effect of Helium and Oxygen Mixtures on Pulmonary Resistances in Emphysema—JOHN M. TYLER, M.D., ELIHU CHANNIN, B.A., AND BIRGER GRAPÉ, M.D., Boston, Mass.

Disturbances of Pulmonary Function in Fifty Patients with Bronchiectasis—NEIL CHERNIACK, M.D., KENNETH L. VOSTI, M.D., GEORGE A. SAXTON, JR., M.D., MARK H. LEPPER, M.D., AND HARRY F. DOWLING, M.D., Chicago, Ill.

Goiter and Myxedema due to Iodide Therapy—BENJAMIN BURROWS, M.D., ALBERT H. NIDEN, M.D., AND WILLIAM R. BARCLAY, M.D., Chicago, Ill.

9:00 a.m.–10:45 a.m.

3B Tuberculosis Research

Aerial Dissemination of Pulmonary Tuberculosis: A Two-Year Study of Contagion in a Tuberculosis Ward—RICHARD L. RILEY, M.D., C. C. MILLS, M.S., AND W. F. WELLS, B.S., Baltimore, Md.

Sites to Which Intranasally Instilled Tubercle Bacilli Are Conveyed—HUGH E. BURKE, M.D., EDITH MANKIEWICZ, M.D., AND JAMES R. STUART, M.D., Montreal, Canada

Tuberculin Allergy in Mice: Comparisons of Mouse Strains and Testing Techniques—ALFRED J. CROWLE, PH.D., Denver, Col.

The Relationship of Serum Levels of a Lysozyme-like Enzyme to Sensitization of Guinea Pigs with

Mycobacterium Tuberculosis—BERNARD W. JANICKI AND ROBERT A. PATNODE, PH.D., Washington, D. C.

The Effect of Fresh Human Serum on the Reaction of Mycobacteriophage D-29 with Certain Mycobacteria—ARTHUR WHITE, M.D., AND VERNON KNIGHT, M.D., Louisville, Ky.

Cytology of Phage-Host Interactions in Mycobacterium Tuberculosis—SMITH SHADOMY, B.A., SEYMOUR FROMAN, PH.D., AND M. JOHN PICKETT, PH.D., Los Angeles, Calif.

The Induction of the Lysogenic State in Mycobacterium Tuberculosis Strain H37Rv—RUTH RUSSEL, B.A., GREGORY JANN, PH.D., AND SEYMOUR FROMAN, PH.D., Los Angeles, Calif.

An Enzymatic Basis for the Potentiation of Isoniazid by Para-aminosalicylic Acid—DAVID A. LEVY, M.D., WILLIAM S. SPICER, M.D., NICHOLAS R. BACHUR, A.B., AND SAMUEL P. BESSMAN, M.D., Baltimore, Md.

The Influence of Isoniazid and Isoniazone Calcium-Pyruvate on the Vitamin B_6 Metabolism of Rats—JENG M. HSU, PH.D., PATRICK B. STOREY, M.D., AND BACON F. CHOW, PH.D., Baltimore, Md.

Discussion

11:00 a.m.–12:00 p.m.

The Amberson Lecture

Viral Infections in Man: Problems and Progress—FRANK L. HORSFALL, JR., M.D., New York, N. Y. JAMES E. PERKINS, *Chairman*; H. WILLIAM HARRIS, *Co-chairman*

12:30 p.m.–2:15 p.m.

American Trudeau Society Luncheon

Program to be announced

2:30 p.m.–4:00 p.m.

4A Respiratory Disease Research

Subjective and Objective Techniques in the Diagnosis of Chronic Bronchitis—CHARLES M. FLETCHER, M.D., London, England

Discussion

Experimental Techniques in Acute Respiratory Disease—ROBERT J. GOODLOW, M.D., Frederick, Md.

The Relationship Between Particle Size and Dose in Respiratory Infections—LEROY D. FOTHERGILL, M.D., Frederick, Md.

Discussion

The Effect of Ultraviolet Radiation upon the Transmission of Epidemic Influenza in Long-term Hospital Patients—ROSS L. MCLEAN, M.D., Atlanta, Ga.

Psittacosis and Q Fever in Laboratory Personnel—ALLAN H. LEVY, M.D., EDWARD W. HOOK, M.D., AND ROBERT R. WAGNER, M.D., Baltimore, Md.

Discussion

2:30 p.m.–4:00 p.m.

4B Experimental Methods

Lung Structure in Three Dimensions after Inflation and Fume Fixation—B. J. BLUMENTHAL AND H. G. BOREN, Houston, Texas

Preparation of Normal and Emphysematous Lung Tissue for Structural Studies of Bronchioles, Alveolar Ducts, and Alveoli—PHILIP C. PRATT, M.D., Columbus, Ohio

Studies of the Elastic Tissue of the Lung—ROBERT W. CARTON, M.D., BEVERLY TEWS, R. N., JOHN DAINAUSKAS, AND GEORGE M. HAAS, M.D., Chicago, Ill.

Discussion

The Erythropoietic Factor in Hypoxic Patients with Emphysema without Secondary Polycythemia—JAMES F. HAMMERSTEN, M.D., WALTER H. WHITCOMB, MC, ROBERT M. BIRD, M.D., PHILIP C. JOHNSON, M.D., AND MARGARET MOORE, B.S., Oklahoma City, Okla.

A Constant Volume Fractional Sample Collector for Repetitive Cardiac Output Determinations—MARGARET E. GANTT, M.D., M. MOSTAFA ABDEL-FATTAH, M.D., AND DAVID W. CUGELL, M.D., Chicago, Ill.

A Rapid Mouse Test for the Diagnosis of Pulmonary Tuberculosis—DAVID GALE, PH.D., ELIZABETH A. LOCKHART, AND ALEXANDER JACK, B.A., Albuquerque, N. M.

2:30 p.m.–4:00 p.m.

4C Tuberculin Testing

PPD Intermediate: Its Diagnostic Significance in Active Tuberculosis—Sol Katz, M.D., Robert C. Duvall, M.D., Thomas J. Ryan, M.D., Thomas F. O'Connor, M.D., R. J. Marilley, M.D., and Richard B. Perry, M.D., Baltimore, Md., and Washington, D. C.

The Variability of Tuberculin Tests—Emil Bogen, M.D., and George Altbach, M.D., Olive View, Calif.

The Tuberculin Test and the Diagnosis of Clinical Tuberculosis—William W. Johnston, B.S., Herbert A. Saltzman, M.D., J. H. Bufkin, C. R. L., and David T. Smith, M.D., Durham, N. C.

Experiences with the Newer Tuberculin Testing Techniques, Patch, Modified Patch, Disk, Heaf, etc.—Michael L. Furcolow, M.D., Lawrence E. Wood, M.D., and Irene L. Doto, M.A., Kansas City, Kansas

Discussion

Comparison between Purified Individual Tuberculoproteins and the International Standard Tuberculin, PPD-S—H. M. Vandiviere, M.A., Florence B. Seibert, Ph.D., Philadelphia, Pa., and M. R. Vandiviere, M.A., Chapel Hill, N. C.

Corticotropin-induced Changes in the Tuberculin Skin Tests: A Controlled Study in Advanced Tuberculosis—Harvey Salomon, M.D., and Joseph Angel, M.B., Brooklyn, N. Y.

Discussion

4:15 p.m.–5:30 p.m.

Case Conference

Gordon Snider, M.D., Chicago, Ill., *Moderator*

Five problem cases will be presented.

4:15 p.m.–5:30 p.m.

Demonstration III

Experimental Techniques for Cardiopulmonary By-pass—F. John Lewis, M.D., Thomas Shields, M.D., and Riccardo Buenvenuto, Chicago, Ill. Limited to 20 physicians. Reservations must be obtained in advance from the American Trudeau Society.

4:15 p.m.–5:30 p.m.

Demonstration IV

Angiocardiography in Pulmonary Disease: Biplane and Cine Equipment—William B. Buckingham, M.D., Gisella Albrecht, M.D., and Aida Baltazaar, Chicago, Ill. Limited to 25 physicians. Reservations must be obtained in advance from the American Trudeau Society.

Wednesday, May 27

9:00 a.m.–11:00 a.m.

5A Long-term Studies in BCG

BCG in Chicago: A Twenty-year Study Statistically Analyzed—Sol Roy Rosenthal, M.D., Erhard Loewinsohn, M.D., Mary L. Graham, Dorothy Liveright, Margaret G. Thorne, Violet Johnson, and H. C. Batson, Chicago, Ill.

Guest speaker to be announced

Discussion

The Evaluation of a Serum Gel-Diffusion Precipitation Test For the Diagnosis of Tuberculosis—Robert C. Parlett, M.D., and Guy P. Youmans, M.D., Chicago, Ill.

A Correlation of the Results of the Agar Double-Diffusion Precipitation Test With the Bacteriological Findings in 189 Consecutive Patients—William Lester, Jr., M.D., and Rosemary Colton, B.A., Hinsdale, Ill.

Discussion

9:00 a.m.–10:00 a.m.

5B Current Concepts in the Surgical Treatment of Cardiac Lesions

Frank L. Gerbode, M.D., San Francisco, Calif., *Moderator*

Acquired Valvular Lesions—ROBERT D. GLOVER, M.D., Philadelphia, Pa.
Congenital Valvular Lesions—DENTON COOLEY, M.D., Houston, Texas
Septal Defects—DONALD B. EFFLER, M.D., Cleveland, Ohio
Anomalous Venous Drainage—THOMAS G. BAFFES, M.D., Chicago, Ill.
Discussion

10:00 a.m.—11:00 a.m.

5C Thoracic Surgery

Histopathology Caused by Atypical Acid-fast Bacilli and Surgical Experience—SUBURBAN COOK COUNTY TUBERCULOSIS-HOSPITAL-SANITARIUM AND UNIVERSITY OF CHICAGO SCHOOL OF MEDICINE
Operative Occlusion of the Bronchus in the Treatment of Selected Cases of Pulmonary Tuberculosis—J. MAXWELL CHAMBERLAIN, M.D., AND THOMAS M. MCNEILL, M.D., New York, N. Y.
Intrathoracic Mesothelioma—DONALD B. EFFLER, M.D., AND CARL A. BROADDUS, JR., M.D., Cleveland, Ohio
Coarctation of the Aorta as Associated with Other Cardiovascular Anomalies—WILLIAM J. KUZMAN, M.D., AND ALBERT B. KLUG, M.D., San Diego, Calif.
Evaluation of Clinical Condition in the Seriously Ill and Critical Effect of Small Overdosage in Medication—RALPH ADAMS, M.D., AND ROBERT CORELL, B.S., Wolfeboro, N. H.
Discussion

11:15 a.m.–12:00 p.m.

The Robert Keeton Memorial Lecture

Chronic Bronchitis: Clinical Characteristics, Pathogenesis, and Pathology—CHARLES M. FLETCHER, M.D., London, England
Introduction by RALPH G. WILLY, M.D., Hinsdale, Ill.

12:30 p.m.–1:45 p.m.

Sandwich Seminar IV*

Differential Diagnosis of Esophageal Disease—WILLIAM E. ADAMS, M.D., Chicago, Ill., *Moderator*
Panelists: E. CLINTON TEXTER, M.D., AND PAUL H. HOLINGER, M.D., Chicago, Ill.

Sandwich Seminar V*

Childhood Tuberculosis—MARGARET H. D. SMITH, M.D., New York, N. Y., *Moderator*
Panelists: ARTHUR ROBINSON, M.D., Denver, Col., and JOHN S. CHAPMAN, M.D., Dallas, Texas

Sandwich Seminar VI*

Radioisotopes in Pulmonary Disease—WILLIAM R. BARCLAY, M.D., Chicago, Ill., *Moderator*
Panelists: LLOYD ROTH, M.D., Chicago, Ill., PAUL E. HAHN, M.D., Nashville, Tenn., AND RICHARD S. FARR, M.D., Pittsburgh, Pa.

2:00 p.m.–4:00 p.m.

6A Symposium on Smoke, Smoking, and Chest Diseases

NORTON NELSON, M.D., New York, N. Y., *Moderator*
Statistical Associations Between Cigarette Smoking and Pulmonary Diseases—E. CUYLER HAMMOND, PH.D., New York, N. Y.
A Critical Examination of Statistical Evidence Linking Cigarette Smoking with Chest Diseases—ABRAHAM M. LILIENFELD, M.D., Baltimore, Md.
The Localization of Inhaled Foreign Matter in the Respiratory Tract—A. C. HILDING, M.D., Minneapolis, Minn.
Pathogenesis of Pulmonary Disease Due to Chemical Environmental Agents—PAUL KOTIN, M.D., Los Angeles, Calif.
An Experimental Approach to the Effects of Tobacco Smoke on the Lungs—CECILIE LEUCHTENBERGER, PH.D., Cleveland, Ohio
Pathological Changes Observed in the Bronchi Associated with Smoking—OSCAR AUERBACH, M.D., East Orange, N. J.
A Critical Examination of Experimental Data on Smoking and Chest Diseases—ROBERT C. HOCKETT, PH.D., New York, N. Y.

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* Each seminar will be limited to 35 physicians in order to allow for informal discussion with the experts. Tickets must be purchased in advance from the American Trudeau Society.

Certain Research Accomplishments and Research Needs in the Lung Cancers—DEAN F. DAVIES, M.D., New York, N. Y.
Discussion

4:15 p.m.–5:30 p.m.

Case Conference
OSCAR AUERBACH, M.D., East Orange, N. J., *Moderator*
Five problem cases will be presented.

4:15 p.m.—5:30 p.m.

Demonstration V
High Energy X-ray Devices—ROBERT MOSELEY, M.D., AND JAMES WILLIAMS, M.D., Chicago, Ill. Limited to 25 physicians. Reservations must be obtained in advance from the American Trudeau Society.

4:15 p.m.–5:30 p.m.

Demonstration VI
Angiocardiography—M. RABINOWITCH, M.D., PETER MOULDER, M.D., AND ROBERT MOSELEY, M.D., Chicago, Ill. Limited to 25 physicians. Reservations must be obtained in advance from the American Trudeau Society.

Obituaries

William Dunn Walker

1920–1957

Dr. William Dunn Walker was born in Charleston, West Virginia, in 1920. After obtaining his medical degree from the University of Virginia, in 1944, Dr. Walker served on the resident staff at the Grant and the Childrens Memorial hospitals in Chicago, Illinois. After an internship at the Great Lakes Naval Hospital he was commissioned as a Lieutenant in the Medical Corps of the United States Navy. In 1945 he was assigned to the Pacific theater, where he served until 1946 when he was separated from the service because of pulmonary tuberculosis contracted while on duty in Northern China.

From 1946 to 1952, Dr. Walker was a patient in several hospitals in New York and Virginia, where he underwent extensive bilateral surgery. His own illness intensified his interest in the field of pulmonary disease and, after his discharge as a patient from the University of Virginia Hospital, he continued as a staff member in the anesthesiology division until a few weeks before his death on October 8, 1958.

Dr. Walker became a member of the American Trudeau Society in 1950. He was also a member of the Elks Lodge in Charleston, West Virginia, and of the Junior Chamber of Commerce in Charlottesville, Virginia. In addition to his professional skills, Dr. Walker had an intense interest in music and was an accomplished pianist as well as a collector of records.

J. D. M.

Edwin Herms Brady

1898–1958

Dr. Edwin H. Brady died of uremia on January 8, 1958, at a hospital in Auburn, California. He was born in Ogdensburg, New Jersey, on March 12, 1898.

He graduated from the University of Oregon, receiving his B.A. in 1938, and from the Creighton University School of Medicine in 1941. Immediately after his internship at the City Hospital, Welfare Island, New York, Dr. Brady entered the Armed Forces, and was discharged in June, 1946.

Subsequently, his career was devoted to tuberculosis, beginning as staff physician at the Eastern Oregon Tuberculosis Sanatorium at The Dalles; he then became the medical director of the Idaho State Tuberculosis Hospital at Gooding, and latterly, from August 1, 1954, he was in charge of the tuberculosis service at the DeWitt State Hospital at Auburn, California.

He is survived by his widow and his daughter.

E. K.

Leonidas F. Barrier

1884–1958

Dr. Leonidas F. Barrier received his medical degree from the Kentucky School of Medicine, Louisville, Kentucky, in 1908. He became interested in internal medicine and pulmonary disease and limited his practice to those specialties.

Dr. Barrier was engaged in private practice in Little Rock, Arkansas, until he retired in February, 1957. He was associated with the Arkansas Baptist Hospital and St. Vincents Infirmary in Little Rock. He was an active member of the American Medical Association and the American Trudeau Society.

Dr. Barrier died on January 9, 1958, of cancer of the bladder.

J. D. M.

John Philip Schantz

1920–1958

Dr. John Philip Schantz of Schuylkill Haven, Pennsylvania, died on January 27, 1958, at the age of thirty-seven. Dr. Schantz obtained his medical degree at Hahnemann Medical College, Philadelphia, in 1946. After internship and residency training in 1946 to 1947, he engaged in general practice at Allentown, Pennsylvania, from 1947 to 1950, when he was recalled to duty with the United States Army. From 1952 to 1955 he served as a staff physician at the Charles Miner Sanatorium in Hamburg, Pennsylvania, and then returned to a general medical practice at Schuylkill Haven, Pennsylvania, where he resided until his death. He was a member of the American Trudeau Society and the American Legion.

Dr. Schantz is survived by his widow, Mildred (Katzaman) Schantz, and two sons, John P. Schantz, Jr., thirteen, and Michael H. Schantz, age ten.

J. D. M.

Grover S. Brzozowski

1886–1958

Dr. Brzozowski died at Lakeside, California, on February 22, 1958, at the age of seventy-one.

Dr. Brzozowski was born at Louisville, Ken-

tucky, in 1886, and graduated from the Louisville Medical School in 1907. During the first World War, he served in the Armed Forces. Remaining then in private practice until 1940, he began his career in tuberculosis at the Stillwater Sanatorium, Dayton, Ohio.

Subsequently, he served at the Battey State Hospital, Rome, Georgia, Piedmont Sanatorium in Virginia, and the William Rush Memorial Hospital in Toledo, Ohio, retiring in 1954.

E.K.

E. F. Sloan

1892–1958

Dr. E. F. Sloan of Columbiana, Alabama, died on June 9, 1958. A native of Alabama, Dr. Sloan obtained his medical degree at Emory University School of Medicine, Atlanta, Georgia, in 1916. He entered into the general practice of medicine in Tuscaloosa and Jefferson County, Alabama, after completion of his training. He became interested in tuberculosis and public health and for more than twenty years had served as Public Health Officer of Shelby County, Alabama.

As a member of the Shelby County Medical Society, he had at one time held every office in that organization. He was Secretary-Treasurer at the time of his death. He was a member of the American Medical Association, the American Trudeau Society, and the Alabama Medical Society. Dr. Sloan was Medical Director of the Shelby County Tuberculosis Association.

A most fitting tribute was contained in a statement authorized by the Shelby County Medical Society: "Dr. Sloan represented to us a truly great master in the practice of the art of medicine as well as the art of living." His high ideals and keen sense of ethical behavior, his understanding of human beings, and his self-discipline made his advice and counsel sought by the members of his profession and of the community.

Dr. Sloan was a member of the First Presbyterian Church of Tuscaloosa, Alabama. He is survived by his widow, Mrs. Helen Sloan.

J. D. M.

Horst Lange

1902–1958

Dr. Horst Lange, a native of Dantzig, Germany, obtained his medical degree from the University of Berlin Medical Faculty in 1924. He served as assistant physician at the Municipal Hospital in Berlin from 1925 to 1931. There he became interested in internal medicine and pulmonary tuberculosis. He entered into private practice in Berlin-Pankow in 1930 and remained until 1933 when he went to Shanghai, China, where he was supervisor of the X-Ray and Tuberculosis Services at the Shanghai Refugee Hospital, and also practiced as an internist. He remained in Shanghai until April, 1951, when he became affiliated with the International Refugee Organization in Italy.

In 1953 he started in general practice at Seaford, Long Island, New York, and continued until his death.

Dr. Lange was a member of the American Trudeau Society, the Chinese Medical Society, the American Medical Association, the American Academy of General Practice, and the Nassau County Medical Society.

He was also interested in fraternal affairs and, as a member of B'nai Brith, the Lions, and of the Masonic Order in China and the United States, contributed to the community programs.

He died on June 15, 1958, at the age of fifty-six, of a cardiovascular accident. He is survived by his widow, Mrs. G. Lange.

J. D. M.

Frank G. Seldon

1899–1958

Dr. Seldon was born in Schniedemuehl, Germany, on June 18, 1899. He graduated from the University of Berlin Medical School in 1923. He served in various capacities in the Rudolph Virchow Hospital and at the Surgical Policlinic of the University of Berlin, and as Assistant Physician at the Tuberculosis Hospital of the City of Berlin.

From January, 1930, to June, 1937, Dr. Seldon practiced as a tuberculosis specialist in Dresden. In 1937 he and his family came to the United States, and in December of that year he accepted an appointment as assistant physician in the New Hampshire State Sanatorium at Glencliff, continuing service in this sanatorium until July, 1942, when he became Medical Superintendant of the Edward Sanatorium at Naperville, Illinois.

In 1946, Dr. Seldon and his family went to Manchester, New Hampshire, where he quickly established himself as a recognized authority in the diagnosis and treatment of diseases of the chest. He continued in private practice as a specialist in tuberculosis in Manchester until his final illness. He served on the staff of the Sacred Heart Hospital and as a consultant to the Notre Dame and Moore General hospitals. Dr. Seldon was a member of the American Trudeau Society, a fellow of the American College of Chest Physicians, and a past president of the New England Chapter of the American College of Chest Physicians. He also held membership in the Hillsborough County and New Hampshire medical societies and the American Medical Association.

A cultured gentleman, Dr. Seldon was widely known for his reading in classical and European literature and as an authority in music and other arts.

Dr. Seldon died on June 29, 1958, after a long illness. He is survived by his wife and son.

R. B. K.

Daniel M. Brumfiel

1890–1958

Dr. Daniel M. Brumfiel died at his home in Saranac Lake, New York, on August 20, 1958, at the age of sixty-eight. Dr. Brumfiel was a native of Indiana. He received his B. A. at Lombard College in 1912, his M.A. from the University of Illinois in 1913, his Ph.D. from the University of Iowa in 1917. There he taught animal biology and later, in the Medical School of Iowa University, anatomy and histology. He was awarded his M.D. at the Johns Hopkins School of Medicine in 1924, following which he was in the Department of Internal Medicine at the Henry Ford Hospital, Detroit, until 1925, when he developed pulmonary tuberculosis. After a short period at Trudeau Sanatorium he returned to the Ford Hospital until a relapse in 1928 forced him to leave. For two years he was associated with the late Dr. Francis B. Trudeau and since then had conducted his own practice in Saranac Lake, specializing in diseases of the heart and lungs.

Dr. Brumfiel was a Fellow of the American College of Physicians and a Diplomate of the Board of Internal Medicine. He was consultant at the Trudeau Sanatorium, Will Rogers Hospital, and the Veterans Administration Hospital, Sunmount.

Dr. Brumfiel always was very active in local affairs, medical and civic. He served as president of the local and county medical societies, the Medical Board of the Saranac Lake General Hospital, Saranac Lake Rehabilitation Guild, and Red Cross. He was Vice-President of the Saranac Lake Society for the Control of Tuberculosis from 1949 to 1952.

Dr. Brumfiel was a member of the American Heart Association and the National Tuberculosis Association. For the last four years he was attending physician at the Essex County Heart Clinic for Children.

He leaves a widow, Katherine Rutledge Brumfiel, whom he married in Fort Dodge, Iowa, on August 28, 1917.

J. N. H.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29

1960—Los Angeles, California—May 15–20

FORTY-FOURTH ANNUAL SESSION OF THE TRUDEAU SCHOOL OF TUBERCULOSIS AND OTHER PULMONARY DISEASES—1959

The Trudeau School of Tuberculosis and Other Pulmonary Diseases, which will hold its Forty-fourth Session from June 8 to 26, 1959, continues to provide a unique opportunity for training in the field of chest diseases. This annual postgraduate course, conducted under the auspices of the Trudeau Foundation and supported by the Hyde Foundation, is able to provide outstanding instruction at a minimal tuition of $100.00 for a three-week session. Attendance at the Trudeau School carries with it some distinction as well as a thorough review for specialization in pulmonary diseases or for work in public health involving tuberculosis.

In addition to the local medical faculty, consisting of some forty doctors from Saranac Lake, Ray Brook State Tuberculosis Hospital, and the Sunmount Veterans Administration Hospital, about thirty of the leading teachers and investigators in the Eastern United States and Canada are brought to Saranac Lake each year to lecture or to conduct seminars in their special fields. The guest list includes such lecturers as Oscar Auerbach, D. V. Bates, Paul Bunn, D. Ewen Cameron, David A. Cooper, Martin M. Cummings, Nicholas D'Esopo, Herbert R. Edwards, Lydia Edwards, Shirley Ferebee, Kirby S. Howlett, Julia Jones, Edith M. Lincoln, Herbert C. Maier, John H. McClement, Jonathan F. Meakins, James E. Perkins, Bram Rose, Oscar A. Sander, David T. Smith, William B. Tucker, Norman J. Wilson, Julius L. Wilson, Herman Wechsler, and T. E. Woodward. Approximately half of the time is devoted to tuberculosis and the other half divided between such subjects as silicosis, pulmonary fibrosis, emphysema, fungal infection, sarcoidosis, pneumonias, and intrathoracic tumors.

In June of each year, the Trudeau School gives its students a chance to combine a great educational experience with an enjoyable holiday in the most beautiful part of the Adirondacks, among pleasant companions. The enrollment is necessarily limited and therefore application should be made early. A few scholarships are available for those who qualify.

All inquiries should be addressed to the Secretary, Trudeau School of Tuberculosis and Other Pulmonary Diseases, Box 500, Saranac Lake, N. Y.

XV CONFERENCE OF INTERNATIONAL UNION AGAINST TUBERCULOSIS

The International Union Against Tuberculosis will hold its XV International Conference in Istanbul, Turkey, September 11–18, 1959. Preliminary programs are available at the Executive Offices of the National Tuberculosis Association.

Since its founding in 1920 and particularly in the past decade, this biennial conference has become an outstanding event and an authoritative source of information for all interested in any aspect of tuberculosis control. As is apparent in the list of scientific sessions below, the scope has broadened to include peripheral topics as well as strictly medical. Our Councillors have been in favor of this expansion as the Union is a federation of voluntary tuberculosis associations and can mitigate toward a better understanding of this concept in every country where an association exists. Several of the members, in addition to having accepted chairmanships will be speakers.

Details of travel, et cetera, can also be procured from the Executive Office of the National Tuberculosis Association, and anyone interested in attending is urged to write for both program and information.

Scientific Sessions

Tuesday, 15th September

9 a.m.–1 p.m.

SYMPOSIUM I: Chemoprophylaxis of tuberculosis—its experimental, clinical and social aspects.

Chairman: Professor Robert Debré (France).

3 p.m.–6 p.m.

SYMPOSIUM II: The problem of acid-fast bacilli (ecology, pathogenicity for man, bacteriologic characteristics).

Chairman: Professor Daniel E. Jenkins (U.S.A.).

Wednesday, 16th September

9 a.m.–1 p.m.

SYMPOSIUM III: Present day indications of surgical treatment of pulmonary tuberculosis.

Chairman: Sir Clement Price Thomas (Great Britain).

3 p.m.–6 p.m.

SYMPOSIUM IV: New antibacterial drugs.

Chairman: Professor L. Hailmeyer (Germany).

Thursday, 17th September

9 a.m.–1 p.m.

SYMPOSIUM V: The problem of relapse in all of its aspects (relapse after chemotherapy, surgery, collapse therapy, ambulatory treatment, and its importance from the epidemiologic standpoint).

a) Relapse after medical treatment:

Chairman: Professor M. Variéty (France).

b) Relapse after surgical treatment:

Chairman: Dr. R. S. Mitchell (U.S.A.).

Friday, 18th September

9 a.m.–1 p.m.

SYMPOSIUM VI: New methods in rehabilitation of the tuberculous.

Chairman: Dr. K. Oppikofer (Switzerland).

3 p.m.–6 p.m.

SYMPOSIUM VII: Health education in the light of new methods of treatment of tuberculosis.

Chairman: Dr. Tevfik Gokçe (Turkey).

SYMPOSIUM VIII: The role of the public health nurse and the social worker in tuberculosis control.

Chairman: Professor Jadwiga Szustrowa (Poland).

A Seminar on "Recent Knowledge on the Mechanism of Acquired Immunity in Tuberculosis," under the chairmanship of Professor R. Cruikshank (Great Britain), will be held on Friday, 18th September, 1959.

The role of BCG vaccination in the different countries of the world will be discussed on Saturday morning, 19th September, under the chairmanship of Professor Armand Frappier (Canada).

NOTE: The names of the members of the different symposiums and of the co-reporters will be announced later.